

(19) World Intellectual Property  
Organization  
International Bureau



(43) International Publication Date  
4 August 2005 (04.08.2005)

PCT

(10) International Publication Number  
**WO 2005/071069 A1**

(51) International Patent Classification<sup>7</sup>: **C12N 7/04**,  
A61K 39/12, C12N 15/62, C07K 14/08, 19/00, C12N  
15/87, 15/86, 5/10

Daniel [ES/ES]; Serrano, 117, E-28006 Madrid (ES).  
**RODRIGUEZ FERNANDEZ-ALBA, Juan Ramón**  
[ES/ES]; Ronda de Poniente, 4-2° C-D, E-28006 Tres  
Cantos - Madrid (ES).

(21) International Application Number:  
PCT/EP2005/000695

(74) Agent: **ARIAS SANZ, Juan**; ABG Patentes, S.L., Orense,  
68, 7th floor, E-28020 Madrid (ES).

(22) International Filing Date: 21 January 2005 (21.01.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
P200400120 21 January 2004 (21.01.2004) ES

(81) Designated States (unless otherwise indicated, for every  
kind of national protection available): AE, AG, AL, AM,  
AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,  
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI,  
GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE,  
KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD,  
MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG,  
PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM,  
TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM,  
ZW.

(71) Applicants (for all designated States except US):  
**CONSEJO SUPERIOR DE INVESTIGACIONES**  
**CIENTÍFICAS** [ES/ES]; Serrano, 117, E-28006 Madrid  
(ES). **BIONOSTRA, S.L.** [ES/ES]; Ronda de Poniente,  
4-2° C-D, E-28760 Tres Cantos - Madrid (ES).

(84) Designated States (unless otherwise indicated, for every  
kind of regional protection available): ARIPO (BW, GH,  
GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM,  
ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),  
European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI,  
FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO,  
SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN,  
GQ, GW, ML, MR, NE, SN, TD, TG).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **RODRIGUEZ**  
**AGUIRRE, José Francisco** [ES/ES]; Serrano, 117,  
E-28006 Madrid (ES). **RUIZ CASTON, José** [ES/ES];  
Serrano, 117, E-28006 Madrid (ES). **GONZALEZ**  
**DE LLANO, María Dolores** [ES/ES]; Serrano, 117,  
E-28006 Madrid (ES). **RODRIGUEZ AGUIRRE, María**  
**Dolores** [ES/ES]; Serrano, 117, E-28006 Madrid (ES).  
**BLANCO CHAPINAL, Soledad** [ES/ES]; Serrano, 117,  
E-28006 Madrid (ES). **OÑA BLANCO, Ana María**  
[ES/ES]; Serrano, 117, E-28006 Madrid (ES). **SAUGAR**  
**GOMEZ, Irene** [ES/ES]; Serrano, 117, E-28006 Madrid  
(ES). **ABAITUA ELUSTONDO, Fernando** [ES/ES];  
Serrano, 117, E-28006 Madrid (ES). **LUQUE BUZO,**

**Published:**

- with international search report
- before the expiration of the time limit for amending the  
claims and to be republished in the event of receipt of  
amendments

For two-letter codes and other abbreviations, refer to the "Guid-  
ance Notes on Codes and Abbreviations" appearing at the begin-  
ning of each regular issue of the PCT Gazette.

(54) Title: CHIMERIC EMPTY CAPSIDS OF THE INFECTIOUS BURSAL DISEASE VIRUS (IBDV), OBTAINMENT  
PROCESS AND APPLICATIONS

(57) Abstract: The chimeric empty capsids of the infectious bursal disease virus (IBDV), are constituted by assembly of (i) IBDV  
pVP2 proteins and (ii) fusion proteins comprising a region A constituted by the IBDV VP3 protein bound to a region B constituted  
by a heterologous polypeptide comprising a polypeptide of interest, such as a polypeptide useful in vaccination, therapy or diagnosis.



WO 2005/071069 A1

## CHIMERIC EMPTY CAPSIDS OF THE INFECTIOUS BURSAL DISEASE VIRUS (IBDV), OBTAINMENT PROCESS AND APPLICATIONS

### FIELD OF THE INVENTION

5       The invention is related to the production of chimeric empty particles of the infectious bursal disease virus (IBDV) and their applications.

### BACKGROUND OF THE INVENTION

10       Viral particles are structures specialized in the packaging and incorporating in vehicles of nucleic acids and proteins. A general feature of viral particles is their excellent ability for the immune response stimulation of the host. These properties make viral particles agents of extraordinary interest for the development both of intracellular delivery systems and for the generation of particulate vaccines. The use of different genetic expression systems has facilitated the production of viral-like particles or empty viral capsids (VLPs) of different types  
15       of viruses (US patent 6,458,362 Casal, et al. 2002. Recombinant VP2 parvoviral pseudo-particles encoding CTL or T-helper cell epitopes; US 5,932,426 Baralle, et al. 1999. Molecular presenting system; US 6,602,705 Barnett, et al. 2003 Expression of HIV polypeptides and production of virus-like particles). The genetic manipulation of these expression systems in turn allows the production of VLPs containing heterologous amino acid sequences coming  
20       from proteins other than those forming the native viral capsid. These VLPs are generically called heterotypical, recombinant or chimeric VLPs (CVLPs). CVLPs have mainly been used for two purposes: (i) generation of multivalent vaccines by means of immunologically relevant heterologous peptides (Kingsman, A. J., N. R. Burns, G. T. Layton, and S. E. Adams. 1995. Yeast retrotransposon particles as antigen delivery systems. *Ann. N. Y. Acad. Sci.* 754: 202–  
25       213; Lo-Man, R., P. Rueda, C. Sedlik, E. Deriaud, I. Casal, and C. Leclerc. 1998. A recombinant virus-like particle system derived from parvovirus as an efficient antigen carrier to elicit a polarized Th1 immune response without adjuvant. *Eur. J. Immunol.* 28: 1401–1407; Qiu, Z., D. Ou, H. Wu, T. C. Hobma, and S. Gillam. 1994. Expression and characterization of virus-like particles containing rubella virus structural proteins. *J. Virol.* 68: 4086–4091); and  
30       (ii) modification of the tropism by means of insertion of amino acid sequences involved in interactions with receptor-ligand (Schmidt, U., Rudolf, R., and Bömh, G. 2001. Binding of external ligands onto an engineered virus capsid. *Prot. Eng.* 14: 769-774; Shin, Y.C., and Folk,

W.R. 2003. Formation of polyoma virus-like particles with different VP1 molecules that bind the urokinase plasminogen activator receptor. *J. Virol.* 77: 11491-11498).

CVLPs are generally obtained by means of the expression of the viral protein(s) responsible for the formation of the viral capsid, fused to the region encoding the polypeptide of interest.

The infectious bursal disease virus (IBDV), belonging to the *Birnaviridae* family, infects different bird species and is directly responsible for a severe immunosuppressive disease causing important economic losses in the world poultry industry.

IBDV particles are icosahedral, with T=13 symmetry, they lack an envelope and are formed by a single protein layer. Up until now, the approaches aimed at obtaining an atomic model for IBDV particles have failed. As a result, the structural information available is based on three-dimensional models generated from images obtained by electron cryomicroscopy of the purified virus and of the VLPs. Based on these studies, it has been verified that the outer surface of the particle is formed by a continuous lattice of 260 trimers of the VP2 protein (37 kDa) organized in five different formations. The inner face of the particles contains 200 trimers of the VP3 protein (29 kDa), the latter, independent from one another, are bound to the basal area of the VP2 trimers. It has been suggested that a third polypeptide, VP4 (28 kDa), could also be part of the particles, being located at the base of the pentamers forming the vertices of the icosahedral structure.

The VP2, VP3 and VP4 polypeptides are produced from the proteolytic processing of a polypeptide precursor of a size of 109 kDa. This precursor is auto-catalytically processed, releasing the pVP2 (48 kDa), VP3 and VP4 polypeptides. The VP4 domain, which is located in the central region of the polyprotein, belongs to the Lon protease family and is responsible for the proteolytic cleavage. The pVP2 and VP3 polypeptides are directly responsible for the capsid assembly. The pVP2 product undergoes a last cleavage at its C-terminal end before giving rise to the mature form of the protein, VP2, which is the one found in purified particles (Da Costa, B., Chevalier, C., Henry, C., Huet, J. C., Petit, S., Lepault, J., Boot, H. & Delmas, B. (2002). The capsid of infectious bursal disease virus contains several small peptides arising from the maturation process of pVP2. *Journal of Virology* 76:2393-2402). This pVP2 processing is necessary for the correct formation of the capsids and requires the presence of VP3, although the responsible protease has not yet been identified (Maraver, A., Oña, A., Abaitua, F., González, D., Clemente, R., Diaz-Ruiz, A., Caston, J. R., Pazos, F. & Rodríguez, J. F. (2003). The oligomerization domain of VP3, the scaffolding protein of

infectious bursal disease virus, plays a critical role for capsid formation. *Journal of Virology* 77:6438-49).

In general terms, morphogenesis is a vital process for the viral cycle requiring successive steps associated to modifications in the polypeptide precursors. As a result, viruses have developed strategies allowing the sequential and correct interaction between each one of their components. One of these strategies, frequently used by icosahedral viruses, is the use of polypeptides coming from a single polyprotein as the base of their structural components. In these cases, the suitable proteolytic processing of said polyprotein plays a crucial role in the assembly process.

This concept for the assembly of IBDV capsids has been demonstrated in earlier work (Fernández-Arias, A., Risco, C., Martínez, S., Albar, J. P. & Rodríguez, J. F. (1998). Expression of ORF A1 of infectious bursal disease virus results in the formation of virus-like particles. *Journal of General Virology* 79, 1047-1054). Expression of the gene encoding for the IBDV polyprotein in eukaryotic cells gives rise to the formation of VLPs that are completely morphologically and biochemically indistinguishable from the IBDV virions. It has also been shown that the assembly of the capsids requires only the synthesis and correct processing of the viral polyprotein and is independent of the presence of the viral genome or of other proteins encoded by the viral genome, such as VP5 and VP1.

The results obtained to date from the IBDV gene expression in different recombinant systems has allowed concluding that: i) the assembly process is independent of the presence of genetic material of the virus, ii) only the polypeptides encoded by the polyprotein gene are necessary for the assembly, and iii) the assembly requires a coordinated interaction between the pVP2 and VP3 polypeptides.

However, it must be indicated that it is not known if the VP2/VP3 interaction is established between VP2 and VP3 domains of the polyprotein precursor when it has not yet undergone modifications, or on the contrary, if this interaction occurs after the processing of the precursor. Furthermore, current information does not exclude the possibility that VP4 could play a relevant role in the morphogenesis of the viral capsid. In fact, IBDV VLPs formed by assembly of the IBDV VP2, VP3 and VP4 proteins have been disclosed (US 6,528,063, US 5,788,970 and JP 5194597).

The work developed by the same inventors has enabled establishing systems for obtaining IBDV VLPs using different eukaryotic expression vectors. These vectors have been used for IBDV polyprotein expression in the absence or presence of the viral VP1 RNA

polymerase. The biochemical characterization of purified VLPs demonstrates that they contain pVP2, VP2 and VP3 proteins when only the viral polyprotein is expressed, and the pVP2, VP2, VP3 and VP1 proteins when the simultaneous expression of the polyprotein and viral RNA polymerase is carried out (Fernández-Arias, A., Risco, C., Martínez, S., Albar, J. P. & Rodríguez, J. F. (1998). Expression of ORF A1 of infectious bursal disease virus results in the formation of virus-like particles. *Journal of General Virology* 79: 1047-1054; Martínez-Torrecuadrada, J. L., Castón, J. R., Castro, M., Carrascosa, J. L., Rodríguez, J. F. & Casal, J. I. (2000). Different architectures in the assembly of infectious bursal disease virus capsid proteins expressed in insect cells. *Virology* 278: 322-331; Maraver, A., *et al.*, (2003) cited *supra*; Lombardo, E., Maraver, A., Castón, J. R., Rivera, J., Fernández-Arias, A., Serrano, A., Carrascosa, J. L. & Rodríguez, J. F. (1999). VP1, the putative RNA-dependent RNA polymerase of infectious bursal disease virus, forms complexes with the capsid protein VP3, leading to efficient encapsidation into virus-like particles. *Journal of Virology* 73: 6973-6983).

On the other hand, patent application WO 02/088339 discloses IBDV viral-like particles formed by assembly of chimeric proteins comprising the IBDV polyprotein bound at its carboxyl terminal end to a polypeptide.

However, CVLPs solely based on IBDV pVP2 and VP3, the latter VP3 protein being fused to a polypeptide of interest, or their potential as vehicles of products of interest, have not been previously disclosed.

## SUMMARY OF THE INVENTION

The invention is faced with the problem of providing new tools for incorporating in vectors or vehicles products of interest, such as molecules with biological activity, for example drugs, polypeptides, proteins, nucleic acids, etc.

The solution provided by this invention is based on it being possible to generate, based on the simultaneous expression of the IBDV pVP2 and VP3 proteins, the latter genetically modified to include a nucleotide sequence encoding for a heterologous polypeptide comprising a polypeptide of interest, IBDV chimeric empty capsids (CVLPs). The resulting CVLPs are formed by assembly of (i) IBDV pVP2 proteins and (ii) fusion proteins comprising a region A constituted by the IBDV VP3 protein bound to a region B constituted by a heterologous polypeptide comprising a polypeptide of interest, wherein said region B is bound to the amino- or carboxy- terminal end of said IBDV VP3 protein. These CVLPs can be used for therapeutic,

preventive or diagnostic purposes, etc., for example in the manufacture of gene therapy vectors or vaccines.

The inventors had previously found that when IBDV VPX (pVP2) and VP3 proteins are expressed from independent genes, empty IBDV particles (VLPs) are formed. These VLPs are structurally identical to those obtained by means of expression of the ORF corresponding to the IBDV polyprotein. As part of the development of new vaccination strategies, the possibility of using this IBDV VLP production strategy for obtaining CVLPs which contained heterologous amino acid sequences, corresponding to peptides of interest, such as a histidine tag (Example 1), GFP (Example 2) and finally peptides involved in immune response induction (Example 3), was analyzed. As is demonstrated, the fusion of heterologous sequences in these constructs is not an obstacle for the formation of CVLPs.

As a study model of peptide-transporting CVLPs involved in an immune response, the possibility of obtaining CVLPs which had the sequence corresponding to the CD8 epitope (E-CD8) of the malaria CS protein (*Plasmodium yoelii*) was approached. This epitope is responsible for the CD8-specific cellular immune response induction against this pathogen, which can be quantified by means of the ELISPOT technique in splenocyte cultures from BALB/c mice (Example 3).

In summary, the obtained results clearly show that: (i) the expression system used allows obtaining IBDV CVLPs containing heterologous amino acid sequences; and (ii) immunization with said IBDV CVLPs induces a specific immune response to the heterologous amino acid sequence present in the CVLPs.

Therefore, an aspect of the present invention is related to an IBDV chimeric empty capsid characterized in that it is constituted by assembly of (i) IBDV pVP2 proteins and (ii) fusion proteins comprising a region A constituted by the IBDV VP3 protein bound to a region B constituted by a heterologous polypeptide comprising a polypeptide of interest.

A further aspect of this invention is related to a process for producing said IBDV CVLPs provided by this invention, based on the gene coexpression of said IBDV pVP2 and fusion proteins as two independent genes.

The nucleic acids, gene constructs, expression systems and host cells developed for implementing said process of producing said IBDV CVLPs, as well as their use for the production of said IBDV CVLPs, constitute further aspects of the present invention.

Said IBDV CVLPs have the ability to incorporate in vectors or vehicles products of interest such as molecules with biological activity, for example, polypeptides, proteins,

nucleic acids, etc. In a particular embodiment, said IBDV CVLPs can internally incorporate in vehicles antigens and immune response inducers in animals or humans to whom it is supplied, whereby they can be used in the manufacture of vaccines against human and animal diseases caused by viruses, bacteria, parasites or any other type of microorganism or against tumor diseases. In another particular embodiment, said IBDV CVLPs are used in the manufacture of gene therapy vectors.

Therefore, in a further aspect, the present invention is related to the use of said IBDV CVLPs in the manufacture of medicaments, such as vaccines and gene therapy vectors. Said vaccines and vectors constitute further aspects of the present invention.

10

### BRIEF DESCRIPTION OF THE FIGURES

**Figure 1.** (a) The diagram schematizes the proteolytic processing steps necessary for the formation of mature VP2 and VP3 capsid proteins from the polyprotein precursor. (b) The diagram reflects different genetic constructs derived from the IBDV polyprotein described up until now, as well as the structures produced by means of its expression in different heterologous systems. The numbers indicate the position corresponding to the first and last amino acid residue of the polyprotein present in each one of the constructs. The lower portion of the figure shows images obtained by means of transmission electron microscopy of the structures obtained by means of expression of the different constructs. The bar corresponds to 50 nm. The data has been taken from the following literature references: Fernández-Arias *et al.*, (1998), cited *supra*; Maraver *et al.*, (2003), cited *supra*; Martínez-Torrecuadrada *et al.*, (2000), cited *supra*; Castón *et al.*, 2001. C terminus of infectious bursal disease virus major capsid protein VP2 is involved in definition of the t number for capsid assembly. *Journal of Virology* 75, 10815-10828.

**Figure 2.** Microscopic analysis of H5 insect cells coexpressing pVP2 and VP3. The pVP2 and VP3 protein subcellular distribution was analyzed by means of confocal immunomicroscopy. Cells infected with the FB/pVP2 (a), FB/VP3 (b), or FBD/pVP2-VP3 (c-e) rBVs were incubated with rabbit anti-pVP2 serum and rat anti-VP3 serum. Then the cells were incubated with goat anti-rabbit IgG serum coupled to Alexa 488 (red) and goat anti-rat IgG serum coupled to Alexa 594 (green). The cores were stained with To-Pro 3 (blue). (e) Overlaying of the images shown in panels (c) and (d). Electron microscopy images corresponding to sections of H5 cells infected with different genetic constructs derived from the IBDV polyprotein. (f) Low-magnification image of an H5 cell infected with

30

a parental Fb virus. The insert corresponds to an enlarged detail of the area indicated by the box. (g) Low-magnification image of an H5 cell infected with the FBD/pVPX-VP3 virus. The insert corresponds to an enlarged detail of the area indicated by the box. (h) High-magnification image of an H5 cell infected with the FBD/pVPX-VP3 virus showing the formation of IBDV structures in detail. (i) High-magnification image of a BSC1 cell infected with the VTLacOI/POLY recombinant vaccine virus showing structures similar to those detected in panel (h). The bars indicate 600 nm (panels f and g) and 200 nm (panels h and i).

**Figure 3. Structural and biochemical characterization of the structures derived from IBDV produced in insect cells coinfecting with the FB/pVP2 + FB/his-VP3 recombinant baculoviruses (rBV).** Cells coinfecting with FB/pVP2 and FB/his-VP3 rBVs, or infected with the FBD/Poly-VP1 or FB/pVP2 virus, were used to purify structures derived from IBDV by means of centrifugation on sucrose gradients. Panels (a), (b), and (c) show transmission electron microscopy images corresponding to fraction 4 of the gradients obtained from infections with FBD/Poly-VP1, FB/pVP2+FB/his-VP3, and FB/pVP2, respectively. Panel (d) shows the results of a Western blot analysis of the sucrose gradients corresponding to the cultures infected with FBD/Poly-VP1 and FB/pVP2+FB/his-VP3, respectively. The total extracts (input) and the different fractions of the sucrose gradients (fraction F1 corresponds to the bottom of the gradient) were analyzed by means of Western blot using specific sera against the IBDV VP1, pVP2, VP3, and VP4 proteins, respectively. The molecular mass of the immunoreactive polypeptides is indicated in kDa.

**Figure 4. Biochemical and structural characterization of IBDV VLPs produced in *S. cerevisiae* transformed with the plasmid pESCURA/pVP2-VP3-GFP.** A *S. cerevisiae* culture transformed with the plasmid pESCURA/pVP2-VP3-GFP was grown at 30°C in a medium supplemented with the inducer galactose. At 18 hours, the culture was harvested and centrifuged. The resulting sediment was processed by means of fractioning in a 25-50% linear sucrose gradient. A) Biochemical analysis of samples corresponding to the sediment before fractioning (T) as well as the different fractions of the sucrose gradient. The samples were analyzed by means of SDS-PAGE and Western blot using specific antibodies against VP3 (anti-VP3) and pVP2 (anti-pVP2) proteins. The arrows indicate the positions of the immunoreactive bands corresponding to the VP3-GFP (61 kDa) and pVP2 (48 kDa) proteins, respectively. B) The structural analysis of the obtained samples was carried out by means of TEM. The image corresponds to a micrograph obtained from an aliquot corresponding to the mixture of fractions 7, 8 and 9 of the sucrose gradient. The sample was



stained with uranyl acetate and observed by means of TEM. The bar corresponds to 65 nm. C) VLPs sample obtained by means of the IBDV polyprotein expression in mammal cells by means of infection with the VT7/Poly recombinant vaccine virus (Fernández-Arias *et al.*, (1998), cited *supra*). The bar corresponds to 65 nm.

5           **Figure 5. Structural and biochemical characterization of QVLPs-CD8.** Panel A shows a TEM image of a sample stained with uranyl acetate corresponding to fraction 4 of a sucrose gradient used for the purification of structures carried out on an insect cell extract coinfecting with the FB/pVP2 and PF/his-CD8-VP3 rBVs. The bar indicates 100 nm. Panel B shows the SDS-PAGE and Western blot analyses carried out with an antibody against VP3  
10 protein, of a sample corresponding to fraction 4 (QVLPs-CD8) of a sucrose gradient used for the purification of structures carried out on an insect cell extract co-infected with the FB/pVP2 and PF/his-CD8-VP3 rBVs (see panel A). A sample of purified IBDV virus (IBDV) was used as a control. The sizes of the molecular mass (MW) markers, as well as the molecular mass estimated for the VP3 and his-CD8-VP3 proteins, were indicated.

15           **Figure 6.- Enhancing effect of the specific anti-malaria CD8 cellular immune response by means of immunization with IBDV CVLPs containing the *Plasmodium yoelii* CD8 epitope.** Groups of 4 mice from the BALB/c strain were intraperitoneally inoculated with 50 µg/mouse of QVLPs-CD8 (group IV) or non-chimeric VLPs (group III). A group was inoculated with VVpJRCS ( $10^7$  pfu/mouse), a recombinant virus expressing the  
20 whole *Plasmodium yoelii* CHITOSAN protein (group II) as a control. 15 days later, the mice of all the groups were intraperitoneally immunized with VVpJRCS ( $10^7$  pfu/mouse). One of the groups received at that time a single dose of the viral vector (group I). 15 days after the second immunization, the animals were sacrificed, the spleen was removed and the ELISPOT was carried out against the malaria CD8 peptide. Panel A shows the image of the  
25 ELISPOT wells carried out with different concentrations of splenocytes obtained from the mice belonging to each one of the groups after their incubation in the presence (+CD8 peptide) or absence (-CD8 peptide) of the CD8 peptide. Panel B shows a graph of the results obtained as a number of specific IFN- $\gamma$ / $10^6$  splenocyte secreting cells.

## 30   **DETAILED DESCRIPTION OF THE INVENTION**

In a first aspect, the invention provides an chimeric empty capsid of the infectious bursal disease virus (IBDV), hereinafter CVLP of the invention, characterized in that it is constituted by assembly of (i) IBDV pVP2 proteins and (ii) fusion proteins comprising a

region A constituted by the IBDV VP3 protein bound to a region B constituted by a heterologous polypeptide comprising a polypeptide of interest.

The term "IBDV", as it is used in the present invention, refers to the different IBDV strains belonging to any of the known serotypes (1 or 2) [by way of illustration, see the review carried out by van den Berg TP, Eterradossi N, Toquin D, Meulemans G., en *Rev Sci Tech* 2000 19: 509-43] and the terms "IBDV pVP2 protein" and "IBDV VP3 protein" refer to the different forms of the pVP2 and VP3 proteins representative of any of the mentioned IBDV strains [NCBI protein databank], according to the definition made by Sánchez and Rodríguez (1999) (Sánchez AB, Rodríguez JF. Proteolytic processing in infectious bursal disease virus: identification of the polyprotein cleavage sites by site-directed mutagenesis. *Virology*. 1999 Sep 15; 262(1):190-199), as well as proteins substantially homologous to said IBDV pVP2 and VP3 proteins, i.e. proteins the amino acid sequences of which have a degree of identity regarding said IBDV pVP2 and VP3 proteins of at least 60%, preferably of at least 80%, more preferably of at least 90% and even more preferably of at least 95%.

The IBDV pVP2 protein present in the CVLP of the invention can be any pVP2 protein representative of any IBDV strain, for example, the full-length pVP2 protein of IBDV Soroa strain [NCBI, access number AAD30136].

The fusion protein present in the CVLP of the invention comprises a region A constituted by the IBDV VP3 protein bound to a region B constituted by a heterologous polypeptide comprising a polypeptide of interest. In a particular embodiment, said region B is bound to the amino-terminal region or to the carboxy-terminal region of said IBDV VP3 protein.

The IBDV VP3 protein, constituting region A of said fusion protein, can be any VP3 protein representative of any IBDV strain, for example, the full-length VP3 protein of IBDV Soroa strain [NCBI, access number AAD30136].

Region B present in said fusion protein is constituted by a heterologous polypeptide comprising a polypeptide of interest. As it is used in the present invention, the term "heterologous polypeptide" refers to a polypeptide not belonging to the native IBDV capsid. The size of the polypeptide of interest can vary within a broad interval, from a few amino acids up to hundreds of amino acids. Said polypeptide of interest can be virtually any polypeptide, regardless of its origin (eukaryotic, prokaryotic, viral, etc.), susceptible to being expressed in a recombinant manner. However, in a particular embodiment said polypeptide of interest is a polypeptide useful in vaccination, therapy or diagnosis, such as an epitope or

determining antigen capable of inducing an immune response in animals and humans against diseases caused by viruses, bacteria, parasites or any other type of microorganism, or against tumor diseases.

In a particular embodiment, said region B comprises a single polypeptide of interest.  
5 However, in another particular embodiment, said region B comprises two or more polypeptides of interest, equal or different, which can be forming tandems.

In a particular embodiment, said fusion protein comprises a region A bound to a single region B. In this case, said region B can be bound to the amino-terminal region of VP3, or alternatively to the carboxy-terminal region of VP3, present in region A. As previously  
10 mentioned region B can contain one or more polypeptides of interest. In a particular embodiment, said region B contains a single polypeptide of interest, whereas in another particular embodiment, said region B comprises two or more different polypeptides of interest.

In another particular embodiment, said fusion protein comprises a region A bound to two regions B, one of them bound to the amino-terminal region of VP3 present in region A and  
15 the other one to the carboxy-terminal region of VP3 present in region A. Said regions B can be equal or different, and each one of them can contain one or more polypeptides of interest, which can be equal to or different from one another. In a specific embodiment, the fusion protein comprises a region A bound to a first region B containing a first polypeptide of interest (B1) and a second region B containing a second polypeptide of interest (B2). Said polypeptides  
20 of interest (B1) and (B2) can be equal or different. In a specific embodiment, said polypeptides of interest (B1) and (B2) are different from one another.

Generally, region A (constituted by the IBDV VP3 protein) is not bound directly to said region B (constituted by the heterologous polypeptide comprising a polypeptide of interest), but rather through a linker polypeptide between said regions A and B. Therefore, if  
25 desired the fusion protein of the invention can further contain a linker polypeptide located between said regions A and B. Advantageously, said linker polypeptide is a peptide with structural flexibility, preferably a polypeptide giving rise to a non-structured domain able to induce an immune response or not. By way of illustration, said flexible peptide can contain repetitions of amino acid residues, particular Gly and Ser residues, or any other suitable  
30 repetition of amino acid residues.

The CVLPs of the invention can be obtained by means of the simultaneous expression of said IBDV pVP2 proteins and said fusion protein comprising said regions A and B, in suitable host cells. Said suitable host cells are cells containing the encoding

nucleotide sequence of said fusion protein comprising regions A and B, and the encoding nucleotide sequence of the IBDV pVP2 protein, either in a single gene construct or in two gene constructs. In a particular embodiment, said suitable host cells are cells that are transformed, transfected or infected with a suitable expression system, such as an expression system comprising a gene construct, wherein said gene construct comprises the nucleotide sequence encoding for said fusion protein comprising regions A and B, and the nucleotide sequence encoding for the IBDV pVP2 protein, or else alternatively with an expression system comprising a first gene construct comprising the nucleotide sequence encoding for said fusion protein comprising regions A and B, and a second gene construct comprising the nucleotide sequence encoding for the IBDV pVP2 protein.

Therefore, in another aspect, the invention provides a nucleic acid, the nucleotide sequence of which comprises the nucleotide sequence encoding for said fusion protein forming part of the CVLPs of the invention and comprising a region A constituted by the IBDV VP3 protein bound to a region B constituted by a heterologous polypeptide comprising a polypeptide of interest, wherein said regions B bound to the amino-terminal region or to the carboxy-terminal region of said IBDV VP3 protein. Optionally, the nucleic acid provided by this invention can contain the nucleotide sequence encoding for IBDV pVP2 if desired. More specifically, in a particular embodiment, the nucleic acid sequence provided by this invention comprises (i) a nucleotide sequence comprising the open reading frame or encoding region corresponding to the IBDV VP3 protein and (ii) a nucleotide sequence comprising the open reading frame or encoding region of one or more heterologous polypeptides comprising one or more polypeptides of interest, and optionally if desired, (iii) a nucleotide sequence comprising the open reading frame or encoding regions corresponding to the IBDV pVP2 protein.

In another particular embodiment, the nucleic acid sequence provided by this invention comprises (i) a nucleotide sequence comprising the open reading frame or encoding region corresponding to the IBDV VP3 protein, (ii) a first nucleotide sequence comprising the open reading frame or encoding region of one or more heterologous polypeptides comprising the open reading frame or encoding region of one or more heterologous polypeptides comprising one or more polypeptides of interest, (ii') a second nucleotide sequence comprising the open reading frame or encoding region of one or more heterologous polypeptides comprising one or more polypeptides of interest, wherein said nucleotide sequence can be equal to or different from each first nucleotide sequence, and

optionally if desired, (iii) a nucleotide sequence comprising the open reading frame or encoding region corresponding to the IBDV pVP2 protein. In this case, one of said first or second nucleotide sequence is operatively bound to 5' end of the nucleotide sequence comprising the open reading frame or encoding region corresponding to the IBDV VP3 protein and the other one is operatively bound to the 3' end of the nucleotide sequence comprising the open reading frame or encoding region corresponding to the IBDV VP3 protein.

As it is used in this description, the term "open reading frame corresponding to the pVP2 protein" or "open reading frame corresponding to the IBDV VP3 proteins" includes, apart from the nucleotide sequences of said open reading frames, other open reading frames analogous to the same encoding frames of the IBDV pVP2 and VP3 proteins. Likewise, the term "open reading frame of one or more heterologous polypeptides comprising one or more polypeptides", includes any encoding nucleotide sequence of said heterologous polypeptide(s) comprising one or more polypeptides of interest. As it is used herein, the term "analogous" intends to include any nucleotide sequence which can be isolated or constructed on the base of the encoding nucleotide sequence of IBDV pVP2 and VP3, for example by means of the introduction of conservative or non-conservative nucleotide replacements, including the insertion of one or more nucleotides, the addition of one or more nucleotides at any of the ends of the molecule, or the deletion of one or more nucleotides at any end or inside of the sequence. Generally, a nucleotide sequence analogous to another nucleotide sequence is substantially homologous to said nucleotide sequence. In the sense used in this description, the expression "substantially homologous" means that at the nucleotide level, the nucleotide sequences in question have a degree of identity of at least 60%, preferably of at least 80%, more preferably of at least 90%, and even more preferably of at least 95%.

In another aspect, the invention provides a gene construct comprising a nucleic acid provided by this invention, i.e. a nucleic acid the nucleotide sequence of which comprises the nucleotide sequence encoding for said fusion protein comprising regions A and B, and optionally the nucleotide sequence encoding for said IBDV pVP2 protein. More specifically, in a particular embodiment, the gene construct provided by this invention comprises a nucleotide sequence comprising (i) a nucleotide sequence comprising the open reading frame or encoding region corresponding to the IBDV VP3 protein and (ii) a nucleotide sequence comprising the open reading frame or encoding region of one or more heterologous polypeptides comprising one or more polypeptides of interest, and optionally if desired, (iii)

a nucleotide sequence comprising the open reading frame or encoding region corresponding to the IBDV pVP2 protein. In another particular embodiment, the gene construct provided by this invention comprises a nucleotide sequence comprising (i) a nucleotide sequence comprising (i) a nucleotide sequence comprising the open reading frame or encoding region corresponding to the IBDV VP3 protein, (ii) a first nucleotide sequence comprising the open reading frame or encoding region of one or more heterologous polypeptides comprising one or more polypeptides of interest, (ii') a second nucleotide sequence comprising the open reading frame or encoding region of one or more heterologous polypeptides comprising one or more polypeptides of interest, wherein said second nucleotide sequence can be equal to or different from said first nucleotide sequence, and optionally if desired (iii) a nucleotide sequence comprising the open reading frame or encoding region corresponding to the IBDV pVP2 protein. In this case, one of said first or second nucleotide sequences is operatively bound to the 5' end of the nucleotide sequence comprising the open reading frame or encoding region corresponding to the IBDV VP3 protein, and the other one is operatively bound to the 3' end of the nucleotide sequence comprising the open reading frame or encoding region corresponding to the IBDV VP3 protein.

In another aspect, the invention provides an expression vector or system selected from:

- a) an expression system comprising a gene construct provided by this invention, operatively bound to transcription, and optionally translation, control elements, wherein said gene construct comprises the nucleotide sequence encoding for said fusion protein comprising regions A and B and the nucleotide sequence encoding for the IBDV pVP2 protein; and
- b) an expression system comprising a first gene construct provided by this invention, operatively bound to transcription, and optionally translation, control elements, wherein said first construct comprises the nucleotide sequence encoding for said fusion protein comprising regions A and B, and a second gene construct operatively bound to transcription, and optionally translation, control elements, comprising the nucleotide sequence encoding for the IBDV pVP2 protein.

In a particular embodiment, the expression system provided by this invention comprises a gene construct comprising (i) a nucleotide sequence comprising the open reading frame or encoding region corresponding to the IBDV VP3, (ii) a nucleotide sequence

comprising the open reading frame or encoding region of one or more heterologous polypeptides comprising one or more polypeptides of interest, and (iii) a nucleotide sequence comprising the open reading frame or encoding region corresponding to the IBDV pVP2 protein, wherein said gene construct is operatively bound to transcription, and optionally translation, control elements.

In another particular embodiment, the expression system provided by this invention comprises a first gene construct, operatively bound to transcription, and optionally translation, control elements, said first gene construct comprising (i) a nucleotide sequence comprising the open reading frame or encoding region corresponding to the IBDV VP3 protein, and (ii) a nucleotide sequence comprising the open reading frame or encoding region of one or more heterologous polypeptides comprising one or more polypeptides of interest, and a second gene construct, operatively bound to transcription, and optionally translation, control elements, said second gene construct comprising a nucleotide sequence comprising the open reading frame or encoding region corresponding to the IBDV pVP2 protein

The transcription, and optionally translation, control elements present in the expression system provided by this invention include promoters, directing the transcription of the nucleotide sequences of interest (pVP2, VP3 and heterologous polypeptide) to which it is operatively linked, and other sequences necessary or suitable for the transcription and its suitable regulation in time and place, for example, start and end signals, cleavage sites, polyadenylation signal, replication origin, transcriptional activators (enhancers), transcriptional silencers (silencers), etc.

Virtually any suitable expression system or vector can be used in the generation of the expression system provided by this invention. By way of illustration, said suitable expression or vector systems can be selected, according to the conditions and needs of each specific case, from plasmids, bacmids, yeast artificial chromosomes (YACs), bacteria artificial chromosomes (BACs), bacteriophage P1-based artificial chromosomes (PACs), cosmids, or viruses, which can further have a heterologous replication origin, for example, bacterial or of yeast, so that it may be amplified in bacteria or yeasts, as well as a marker usable for selecting the transfected cells different from the gene or genes of interest. These expression systems or vectors can be obtained by conventional methods known by persons skilled in the art [Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989). Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory] and form part of the present invention. In a particular embodiment, said expression or vector system is a plasmid, such as

a plasmid suitable for transforming yeasts, for example, the plasmid called pESCURA/pVP2-VP3-GFP (Example 2), or a virus, such as a recombinant baculovirus (rBV), for example, the rBV called FBD/pVP2-his-VP3 (Example 1.2), simultaneously expressing both proteins (IBDV pVP2 and his-VP3) in insect cells during the replication cycle, or the rBVs called FB/pVP2 and FB/his-VP3 (Example 1.1) expressing the IBDV pVP2 and his-VP3 proteins, respectively, when coinfecting insect cells, obtaining IBDV CVLPs with the six histidine (6 his) heterologous polypeptide, or the rBVs called FB/pVP2 and FB/his-CD8-VP3 (Example 3) expressing IBDV pVP2 proteins and his-CD8-VP3, respectively, when co-infecting insect cells, forming the capsids called CD8-CVLPs.

In another aspect, the invention provides a host cell containing the encoding nucleotide sequence of said fusion protein comprising regions A and B, and the encoding nucleotide sequence of the IBDV pVP2 protein, either in a single gene construct or in two different gene constructs. In a particular embodiment, said host cell is a host cell that is transformed, transfected or infected with (i) an expression system provided by this invention comprising either a gene construct wherein said gene construct comprises the nucleotide sequence encoding for said IBDV pVP2 protein and the nucleotide sequence encoding for said fusion protein comprising regions A and B, and the encoding nucleotide sequence of the IBDV pVP2 protein, or else alternatively with (ii) an expression system comprising a first construct comprising the nucleotide sequence encoding for said fusion protein comprising regions A and B, and second gene construct comprising the nucleotide sequence encoding for said IBDV pVP2 protein.

In a particular embodiment, the host cell provided by this invention is a host cell that is transformed, transfected or infected with an expression system comprising a gene construct comprising (i) a nucleotide sequence comprising the open reading frame or encoding region corresponding to the IBDV VP3 protein, (ii) a nucleotide sequence comprising the open reading frame or encoding region of a heterologous polypeptide comprising a polypeptide of interest, and (iii) a nucleotide sequence comprising the open reading frame or encoding region corresponding to the IBDV pVP2 protein, wherein said gene construct is operatively bound to transcription, and optionally translation, control elements.

In another particular embodiment, the host cell provided by this invention is a host cell that is transformed, transfected or infected with (a) a first gene construct, operatively bound to transcription, and optionally translation, control elements, said first gene construct



comprising (i) a nucleotide sequence comprising the open reading frame or encoding region corresponding to the IBDV VP3 protein, and (ii) a nucleotide sequence comprising the open reading frame or encoding region of a heterologous polypeptide comprising a polypeptide of interest, and with (b) a second gene construct, operatively bound to transcription, and optionally translation, control elements, said second gene construct comprising a nucleotide sequence comprising the open reading frame or encoding region corresponding to the IBDV pVP2 protein.

Virtually any host cell susceptible to being transformed, transfected or infected by an expression system provided by this invention can be used, for example, mammal cells, bird cells, insect cells, yeasts, etc; however, in a particular embodiment, said host cell is selected from yeasts and insect cells. Yeasts are suitable due to the simplicity and production cost. Insect cells are suitable when the expression system comprises one or two recombinant baculoviruses (rBV). The use of rBV is advantageous due to biosafety issues related to the host range of the baculoviruses, incapable of replicating in other cell types which are not insect cells.

In a particular embodiment, the invention provides a host cell, such as a yeast, for example, *Saccharomyces cerevisiae*, *Saccharomyces pombe*, etc., transformed with an expression system, such as a plasmid or an expression vector, comprising a gene construct provided by this invention comprising the nucleotide sequence encoding for said fusion protein comprising regions A and B, and the nucleotide sequence encoding for the IBDV pVP2 protein.

In another particular embodiment, the invention provides a host cell, such as an insect cell, infected with an expression system, such as a recombinant baculovirus, comprising a gene construct provided by this invention comprising the nucleotide sequence encoding for said fusion protein comprising regions A and B, and the nucleotide sequence encoding for the IBDV pVP2 protein.

In another particular embodiment, the invention provides a host cell, such as an insect cell, coinfecting with an expression system comprising a first recombinant baculovirus comprising a gene construct provided by this invention comprising the nucleotide sequence encoding for said fusion protein comprising regions A and B, and with a second recombinant baculovirus comprising a gene construct provided by this invention comprising the nucleotide sequence encoding for the IBDV pVP2 protein.

In another aspect, the invention provides a process for the production of CVLPs of the invention comprising culturing a host cell provided by this invention containing the encoding nucleotide sequence of said fusion protein comprising regions A and B, and the encoding nucleotide sequence of IBDV pVP2, either in a single gene construct or in two different gene constructs, and simultaneously expressing said IBDV pVP2 proteins and fusion protein comprising regions A and B, and if desired, recovering said CVLPs of the invention. In a particular embodiment, said host cell provided by this invention is a cell that is transformed, transfected or infected with a suitable expression system, such as an expression system comprising a gene construct, wherein said gene construct comprises the nucleotide sequence encoding for said fusion protein comprising regions A and B, and the nucleotide sequence encoding for IBDV pVP2, or else alternatively with an expression system comprising a first gene construct comprising the nucleotide sequence encoding for said fusion protein comprising regions A and B, and a second gene construct comprising the nucleotide sequence encoding for IBDV pVP2.

Said process therefore comprises the gene coexpression of said IBDV pVP2 proteins and fusion proteins comprising regions A and B as two independent genes. After the simultaneous expression of said proteins (IBDV pVP2 and fusion proteins comprising regions A and B) in said cells, the expressed proteins are assembled and form the CVLPs of the invention, which can be isolated or withdrawn from the medium and purified if desired. The isolation and purification of said CVLPs of the invention can be carried out by conventional methods, for example, by means of fractioning on sucrose gradients.

In a particular embodiment, the simultaneous gene coexpression of IBDV pVP2 proteins and fusion proteins comprising regions A and B is carried out by means of the use of an rBV allowing the simultaneous expression of said proteins from two independent chimeric genes in insect cells. In this case, the process for the production of CVLPs of the invention provided by this invention comprises, first the obtainment of a gene expression system constituted by an rBV containing a gene construct simultaneously encoding for the IBDV pVP2 proteins and for said fusion proteins comprising regions A and B, such as the rBV called FBD/pVP2-his-VP3 (Example 1.2), or alternatively the obtainment of an rBV containing a gene construct encoding for the IBDV pVP2 protein and the obtainment of another rBV containing a gene construct encoding for said fusion protein comprising regions A and B, such as the rBVs called FB/pVP2 and FB/his-VP3 (Example 1.1), or rBVs called FB/pVP2 and FB/his-CD8-VP3 (Example 3), respectively, followed by the infection of insect cells with said

expression system based on said recombinant baculovirus(es), expression of the recombinant proteins and if desired, isolation of the CVLPs of the invention formed by assembly of said IBDV pVP2 proteins and fusion proteins comprising regions A and B, and optionally subsequent purification of said CVLPs of the invention.

- 5           The construction of a recombinant baculovirus allowing the independent expression of the IBDV pVP2 proteins and the fusion proteins comprising regions A and B can be carried out by any person skilled in the art based on that described herein and on the state of the art concerning this technology (Cold Spring Harbor, N.Y.; Leusch MS, Lee SC, Olins PO. 1995. A novel host-vector system for direct selection of recombinant baculoviruses (bacmids) in
- 10 *Escherichia coli*. Gene 160: 191-4; Luckow VA, Lee SC, Barry GF, Olins PO. 1993. Efficient generation of infectious recombinant baculoviruses by site-specific transposon-mediated insertion of foreign genes into a baculovirus genome propagated in *Escherichia coli*. J Virol 67: 4566-79).

- In another particular embodiment, the gene coexpression of the IBDV pVP2 proteins
- 15 and of the previously defined fusion proteins comprising regions A and B is carried out by means of the use of a vector allowing the expression of said proteins in yeast cells. In this case, the process for the production of CVLPs of the invention provided by this invention comprises, first, the obtainment of a gene expression system constituted by a plasmid containing a gene construct simultaneously encoding for the IBDV pVP2 proteins and for said fusion proteins
- 20 comprising regions A and B, followed by the transformation of yeasts with said expression system, expression of the recombinant proteins, and if desired, isolation of the CVLPs of the invention formed by assembly of said IBDV pVP2 proteins and fusion proteins comprising regions A and B, and optionally subsequent purification of said CVLPs of the invention. In a specific embodiment, the suitable expression system for transforming yeasts is based on a
- 25 pESC Yeast (Stratagene) expression system such as, for example, the plasmid pESCURA/pVP2/VP3-GFP (Example 2) containing a gene construct encoding for the IBDV pVP2 and VP3-GFP proteins.

- The obtainment of yeasts transformed with a gene construct or with a suitable expression system or vector allowing the simultaneous expression of the IBDV pVP2 proteins
- 30 and the fusion proteins comprising regions A and B can be carried out by any person skilled in the art based on that described herein and on the state of the art concerning this technology (pESC epitope tagging vectors Instructions manual. Stratagene [www.stratagene.com](http://www.stratagene.com);

Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989). Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory).

In another aspect, the invention is related to the use of the gene expression system provided by this invention for producing and obtaining the CVLPs of the invention.

5       The CVLPs of the invention can be used as vectors or vehicles of products of interest, such as molecules with biological activity, for example, drugs, polypeptides, proteins, nucleic acids, etc., whereby they can be used for therapeutic or diagnostic or research purposes. In a particular embodiment, said molecules of biological interest include polypeptides of interest, such as antigens or immune response inducers in animals or humans to whom they are  
10       supplied, or nucleic acid sequences, useful in gene therapy, intended for being introduced inside the suitable cells.

Therefore, in another aspect, the invention is related to the use of the CVLPs of the invention in the manufacture of medicaments, for example vaccines, gene therapy vectors (delivery systems), etc. In a particular embodiment, said medicament is a vaccine intended for  
15       conferring protection against human or animal diseases caused by viruses, bacteria, parasites, or any other type of microorganism, or against tumor diseases. In another particular embodiment, said medicament is a gene therapy vector.

In another aspect, the invention provides a vaccine comprising a therapeutically effective amount of CVLPs of the invention, optionally together with one or more  
20       pharmaceutically acceptable adjuvants and/or vehicles. Said vaccine is useful for protecting animals and humans against diseases caused by microorganisms (viruses, bacteria, parasites, etc.), or against tumor diseases. In a particular embodiment, said vaccine is especially useful for simultaneously protecting animals or humans against the infection caused by two or more infectious disease-causing agents. By way of illustration, the vaccine provided by this  
25       invention can be used to protect birds, for example chickens, turkeys, geese, pheasants, quails, ostriches, etc., against the infectious bursal disease virus (IBDV) and against one or more infectious agents responsible for avian diseases (avian pathogens).

In the sense used in this description, the expression "therapeutically effective amount" refers to the amount of CVLPs of the invention calculated for producing the desired effect and  
30       will generally be determined, among others, by the characteristics of the CVLPs and the immunization effect to be achieved.

The pharmaceutically acceptable adjuvants and vehicles which can be used in said vaccines are those adjuvants and vehicles known by the persons skilled in the art and

normally used in the manufacture of vaccines.

In a particular embodiment, said vaccine is prepared in form of an aqueous solution or suspension in a pharmaceutically acceptable diluent, such as saline solution, phosphate-buffered saline solution (PBS), or any other pharmaceutically acceptable diluent.

5       The vaccine provided by this invention can be administered by any suitable administration route which results in a protective immune response against the heterologous sequence or epitope used, to which end said vaccine will be formulated in the dosage form suited to the chosen administration route. In a particular embodiment, the administration of the vaccine provided by this invention is carried out parenterally, for example,  
10       intraperitoneally, subcutaneously, etc.

The following Examples illustrate the invention and should not be considered limiting of the scope thereof.

## EXAMPLE 1

### 15       Obtaining IBDV CVLPs in insect cells

#### 1.1 Obtaining IBDV CVLPs, VP2-his-VP3, by means of two independent rBVs in insect cells

The results of a series of experiments designed to analyze the possibility of obtaining  
20       IBDV CVLPs from the coexpression of the IBDV pVP2 and VP3 proteins and a heterologous polypeptide from two independent chimeric genes are described in this example. To that end, two recombinant baculoviruses (rBVs) described above, FB/his-VP3 (Kochan, G., González, D. & Rodríguez, J. F. (2003). Characterization of the RNA binding activity of VP3, a major structural protein of IBDV. *Archives of Virology* 148, 723-744) and  
25       FB/VPX, herein cited as FB/pVP2, (Martínez-Torrecuadrada, J. L., Castón, J. R., Castro, M., Carrascosa, J. L., Rodríguez, J. F. & Casal, J. I. (2000). Different architectures in the assembly of infectious bursal disease virus capsid proteins expressed in insect cells. *Virology* 278, 322-331) have been used. These rBVs were generated by means of the cloning into suitable vectors of the complementary DNA (cDNA) encoders of the IBDV pVP2 and pVP3  
30       proteins. Said cDNAs were obtained by RT-PCR from the A segment of the serotype I IBDV Soroa strain genome a (NCBI access number AAD30136). The rBV FB/his-VP3 expresses a chimeric VP3 protein which at its N-terminal end contains a tandem of six histidines fused to the VP3 sequence (Met754-Glu1012 of the polyprotein) called his-VP3. rBV FB/pVP2 expresses the encoding region of the pVP2 protein (Met1-Ala512).

The analysis of the expression of these pVP2 and his-pVP3 proteins, whether independently or together, was carried out in cell cultures. To carry out these experiments, single layer cell cultures from the insect *Trichoplusia ni* (H5, Invitrogen) were used, which were grown on cover glasses. Said cultures were independently infected with FB/pVP2, 5 FB/his-VP3, or coinfecting with both rBVs. The multiplicity of infection was 5 pfu/cell. The cells were fixed at 48 hours post-infection (h.p.i.), and incubated with rabbit anti-VP2 polyclonal serum and with rat anti-VP3 polyclonal serum (Fernández-Arias, A., Risco, C., Martínez, S., Albar, J. P. & Rodríguez, J. F. (1998). Expression of ORF A1 of infectious bursal disease virus results in the formation of virus-like particles. *Journal of General* 10 *Virology* 79, 1047-1054). After successive washings, the cover glasses were incubated with goat anti-rat serum conjugated with Alexa 594 and goat anti-rabbit serum conjugated with Alexa 488 (Jackson ImmunoResearch Laboratories, Inc.). The cellular cores were stained with the specific To-Pro-3 marker (Molecular Probes, Inc.). The samples were finally viewed by epifluorescence with a Zeiss Axiovert 200 microscope equipped with the Bio Rad 15 Radiance 2100 confocal system. The images obtained were stored using the Laser Sharp Package (Bio Rad) software equipment. As is shown in Figure 2a, in the cultures infected with FB/pVP2, the anti-VP2 serum showed a fine granular signal mixed with tubular structures, both distributed throughout the cytoplasm. The anti-VP3 signal, detected in the cells infected with rBV FB/his-VP3, was characterized by the presence of spherical-shaped, 20 and apparently hollow, accumulations around the core. In the cultures coinfecting with both rBVs, a notable modification in the distribution pattern of both proteins was detected. In these cells, the specific signals of pVP2 and VP3 were collocated in spherical and dense accumulations, suggesting that their coexpression allowed the formation of pVP2/his-VP3 complexes (Figure 2c to 2e).

25 For the purpose of characterizing these structures in greater detail, similar extracts corresponding to cells infected with FB/pVP2+FB/hisVP3 were analyzed by transmission electron microscopy (TEM). As a control, and in parallel, H5 cell cultures infected with the wild strain of the FBD (FastBacDual, Invitrogen) virus were analyzed by the same technique. After the infection, the cells were harvested after 48 hours, and processed as has 30 been previously described (Fernández-Arias A, Risco C, Martínez S, Albar JP & Rodríguez JF. (1998). Expression of ORF A1 of infectious bursal disease virus results in the formation of virus-like particles. *Journal of General Virology* 79:1047-1054) for their analysis in ultrathin sections by TRANSMISSION ELECTRON MICROSCOPY. As is shown in Figure

2, the cytoplasm of the coinfecting cells contains aggregates formed by a mixture of tubules and structures similar to capsids (Figure 2g, 2h and 2i). These aggregates were not observed in any case in the samples corresponding to cells infected with wild FBD virus (Figure 2f). The appearance and size of the tubules, as well as of the structures similar to capsids, was similar to those previously described in cell cultures infected with VT7/Poly, a recombinant of the vaccinia virus expressing the gene of the IBDV polyprotein (Fernández-Arias A, Risco C, Martínez S, Albar JP & Rodríguez JF. (1998). Expression of ORF A1 of infectious bursal disease virus results in the formation of virus-like particles. *Journal of General Virology* 79:1047-1054).

To unmistakably establish that the coexpression of pVP2 and his-VP3 enabled the assembly and, therefore, the obtainment of CVLPs, the decision was made to purify the formed particles. To that end, H5 cell cultures were infected with FB/pVP2+FB/his-VP3. At 60 h.p.i., the cells were homogenized and the extracts were separated on sucrose gradients as previously described (Lombardo E, Maraver A, Castón JR, Rivera J, Fernández-Arias A, Serrano A, Carrascosa JL & Rodríguez JF. (1999). VP1, the putative RNA-dependent RNA polymerase of infectious bursal disease virus, forms complexes with the capsid protein VP3, leading to efficient encapsidation into virus-like particles. *Journal of Virology* 73:6973-6983). After their centrifugation, the gradients were fractioned, and the different fractions were analyzed by TEM as previously described (Lombardo E, Maraver A, Castón JR, Rivera J, Fernández-Arias A, Serrano A, Carrascosa JL & Rodríguez JF. (1999). VP1, the putative RNA-dependent RNA polymerase of infectious bursal disease virus, forms complexes with the capsid protein VP3, leading to efficient encapsidation into virus-like particles. *Journal of Virology* 73:6973-6983). As a control, and subject to the same process, gradients corresponding to cell extracts infected with rBV FB/VPX or with rBV FBD/Poly-VP1, were fractioned. The recombinant virus FBD/Poly-VP1 simultaneously expresses the VP1 polypeptide and polyprotein. As was predictable, the infection with FBD/Poly-VP1 had a result of an efficient production of VLPs (Maraver A, Oña A, Abaitua F, González D, Clemente R, Diaz-Ruiz A, Castón JR, Pazos F & Rodríguez JF. (2003). The oligomerization domain of VP3, the scaffolding protein of infectious bursal disease virus, plays a critical role for capsid formation. *Journal of Virology* 77:6438-49). On the other hand, the fractions corresponding to the cells infected with FB/VPX only contain tubules of a twisted appearance. The gradients corresponding to cells coinfecting with the rBVs FB/pVP2+FB/his-VP3 contain rigid type I tubules in the fractions near the bottom of the

gradient, and CVLPs in the central and top fractions (Figure 3b). The CVLPs isolated from the cells coinfecting with rBV FB/pVP2+FB/his-VP3 had a diameter of 65-70 nm, as well as a typical polygonal contour, absolutely indistinguishable from the purified VLPs of cultures infected with FBD/Poly-VP1 (Maraver, A., Oña, A., Abaitua, F., González, D., Clemente, R., Diaz-Ruiz, A., Caston, J. R., Pazos, F. & Rodríguez, J. F. (2003). The oligomerization domain of VP3, the scaffolding protein of infectious bursal disease virus, plays a critical role for capsid formation. *Journal of Virology* 77:6438-49) or of the cultures infected with VT7/Poly (Fernández-Arias, A., Risco, C., Martínez, S., Albar, J. P. & Rodríguez, J. F. (1998). Expression of ORF A1 of infectious bursal disease virus results in the formation of virus-like particles. *Journal of General Virology* 79, 1047-1054).

For the purpose of achieving a biochemical characterization of the obtained material, Western blot experiments were carried out in which the different fractions were compared with specific sera against the VP1, pVP2, VP3 and VP4 proteins (Fernández-Arias *et al.* 1998, cited *supra*; Lombardo *et al.*, 2000). Cell extracts infected with IBDV were used as a control. The obtained results are shown in Figure 3d. As was expected, the bands corresponding to the VP1 and VP4 polypeptides were only detected in samples corresponding to cells infected with FBD/Poly-VP1. The patterns corresponding to pVP2/VP3 in samples corresponding to cells infected with FBD/Poly-VP1 or coinfecting with FB/VPX+ FB/his-VP3 were similar, two bands corresponding to pVP2 and VP3, respectively, being detected.

### 1.2 Obtaining IBDV CVLPs, pVP2-his-VP3, by means of a single rBV in insect cells

Furthermore, the construction of the plasmid pFBD/pVP2-his-VP3 was carried out. The first step of the construction was carried out by means of the cloning of the encoding region of the pVP2 protein into the pFBDual vector (Invitrogen). The DNA fragment corresponding to pVP2 was obtained by means of PCR with the oligonucleotides identified as Oligo I (SEQ ID NO: 1) and Oligo II (SEQ ID NO: 2) using the plasmid pVOTE.2/Poly as a mold (Fernández-Arias, A., Risco, C., Martínez, S., Albar, J. P. & Rodríguez, J. F. (1998). Expression of ORF A1 of infectious bursal disease virus results in the formation of virus-like particles. *Journal of General Virology* 79, 1047-1054). The fragment was purified, subjected to digestion with the BglII and HindIII enzymes and cloned into the pFBDual vector (Invitrogen) previously digested with the BamHI and HindI enzymes. The resulting plasmid was called pFBD/pVP2. Then, a DNA fragment containing the open reading frame



corresponding to the VP3 protein was obtained by means of digestion of the plasmid pFB/his-VP3 (Kochan et al., 2003, cited *supra*) with the RsrII enzyme, treatment with Klenow, and subsequent restriction with KpnI. This DNA fragment was purified and cloned into the plasmid pFBD/pVP2 previously digested with the SmaI and KpnI enzymes. The resulting plasmid was called pFBD/pVP2-his-VP3 (SEQ ID NO: 3) and contains the encoding nucleotide sequence of the pVP2 proteins and of the his-pVP3 fusion protein containing a heterologous his 6 sequence (the latter is encoded by the complementary chain to the nucleotides 6734-7585 of SEQ ID NO: 3). The amino acid sequence of the pVP2 protein and of the his-VP3 fusion protein (pVP2-his-VP3) encoded by the nucleotide sequence contained in said plasmid pFBD/pVP2-his-VP3 is shown in SEQ ID NO: 4.

The plasmid pFBD/pVP2-his-VP3 allows obtaining an rBV, called FBD/pVP2-his-VP3, expressing both proteins simultaneously during its replication cycle [<http://invitrogen.com/content/sfs/manuals/bevtest.pdf>].

The results obtained with FBD/pVP2-his-VP3 in insect cells are identical to those obtained by means of the coinfection with rBVs FB/pVP2 and FD/his-VP3, IBDV CVLPs with the heterologous six histidine (6 his) polypeptide being obtained.

## EXAMPLE 2

### Obtaining IBDV CVLPs, pVP2-VP3-GFP, in yeasts

For the purpose of studying the possibility of obtaining IBDV CVLPs in yeast cultures (*Saccharomyces cerevisiae*) the vector pESCURA/pVP2-VP3-GFP was generated with the heterologous GFP gene bound to the VP3 N-terminal end. The first step in the construction of the vector was carried out by means of the cloning of the encoding region of the pVP2 protein into the vector pESCURAinv. The plasmid pESCURAinv was generated by means of digestion of the vector pRS426 (Stratagene) with the PvuII enzyme and religation of the digestion mixture. The resulting vector, pESCURAinv, contains the multiple cloning region in reversed position with regard to that of parent vector pRS426. The DNA fragment corresponding to the pVP2 protein was obtained by means of PCR with the oligonucleotides called Oligo III (SEQ ID NO: 5) and Oligo IV (SEQ ID NO: 6) using the plasmid pVOTE.2/Poly as a mold (Fernández-Arias, A., Risco, C., Martínez, S., Albar, J. P. & Rodríguez, J. F. (1998). Expression of ORF A1 of infectious bursal disease virus results in the formation of virus-like particles. *Journal of General Virology* 79, 1047-1054). The fragment was purified subjected to digestion with the BglII and HindIII enzymes and cloned

into the vector pESCURA.inv, previously digested with the BamHI and HindIII enzymes. The resulting plasmid was called pESCURA/pVP2.

5 The plasmid pFB/VP3-GFP was constructed in two stages. The first one consisted of the cloning of a DNA fragment, generated by means of PCR, containing the ORF of the VP3 protein lacking the termination codon. This PCR was carried out using the oligonucleotides called Oligo V (SEQ ID NO: 9) and Oligo VI (SEQ ID NO: 10) and using the plasmid pVOTE.2/Poly as a mold (Fernández-Arias, A., Risco, C., Martínez, S., Albar, J. P. & Rodríguez, J. F. (1998). Expression of ORF A1 of infectious bursal disease virus results in the formation of virus-like particles. *Journal of General Virology* 79, 1047-1054). The  
10 resulting DNA was digested with the EcoRI and BamHI enzymes and cloned into the vector pEGFP-N3 (Clontech), also digested with the same enzymes. The resulting plasmid was called pVP3-GFP. Then, the plasmid pEGFP-GFP was digested with the EcoRI and NotI enzymes and cloned into the vector pFastBac1 (Invitrogen). The resulting plasmid was called pFB/VP3-GFP.

15 Next, a DNA fragment that contained the open reading frame corresponding to the VP3 protein fused to the encoding region of the EGFP protein was obtained by means of digestion of the plasmid pFB/VP3-GFP with the EcoRI and NotI enzymes. This DNA fragment was purified and cloned into the plasmid pESCURA/pVP2 previously digested with the EcoRI and NotI enzymes. The resulting plasmid was called pESCURA/pVP2-VP3-  
20 GFP (SEQ ID NO: 7) and contains the ORFs of the pVP2 and VP3-GFP proteins under the transcriptional control of two independent promoters, GAL 1 and GAL 10, both inducible by galactose (the pVP2 protein is encoded by the chain of nucleotides complementary to the nucleotides 5862-7343 of SEQ ID NO: 7). The amino acid sequence of the pVP2 protein and of the VP3-GFP fusion protein (pVP2-VP3-GFP) encoded by the nucleotide sequence  
25 contained in said plasmid pESCURA/pVP2-VP3-GFP is shown in SEQ ID NO: 8.

pESCURA/pVP2-VP3-GFP was subsequently used to transform a culture of *S. cerevisiae* yeast haploid strain 499 according to a previously described protocol (Gietz, R.D. and R.A. Woods. (2002), Transformation of yeast by the Liac/SS carrier DNA/PEG method. *Methods in Enzymology* 350:87-96). The yeasts transformed with the plasmid were selected  
30 by means of growth on SC medium plates (CSM + YNB, 2% glucose and bacto agar) supplemented with the amino acids tryptophan, leucine and histidine and lacking uracil (-Ura). After an incubation of 48 hours at 30°C, a colony was chosen which was used to carry out the following protein expression and CVLP formation analyses.

The pVP2 and VP3 protein expression and CVLP formation analyses were carried out following a protocol previously described for the characterization of IBDV VLPs in other expression systems (Fernández-Arias, A., Risco, C., Martínez, S., Albar, J. P. & Rodríguez, J. F. (1998). Expression of ORF A1 of infectious bursal disease virus results in the formation of virus-like particles. *Journal of General Virology* 79, 1047-1054; Lombardo, E., Maraver, A., Castón, J. R., Rivera, J., Fernández-Arias, A., Serrano, A., Carrascosa, J. L. & Rodríguez, J. F. (1999). VP1, the putative RNA-dependent RNA polymerase of infectious bursal disease virus, forms complexes with the capsid protein VP3, leading to efficient encapsidation into virus-like particles. *Journal of Virology* 73, 6973-698). The colony selected was cultured in liquid CSM (-Ura) + YNB medium supplemented with 2% raffinose. The culture was incubated at 30°C for 24 hours. This culture was used to inoculate, at an optical density (O.D.) of 0.2, a flask of 200 ml of CSM (-Ura) + YNB medium supplemented with 2% inducer galactose. The culture was maintained at 30°C for 18 hours (until an O.D. between 1.0 and 2.0). The yeasts were centrifuged at 3,000 *g* for 5 minutes, 5 minutes at 4°C, were washed once with distilled water, and the pellet was resuspended in lysis buffer (TEN: Tris 10 mM, pH 8.0; NaCl 150 mM; EDTA 1 mM) + 2X protease inhibitors (Compl Roche). A volume of glass beads having a size of about 425-600 microns (Sigma) were added for the lysis. This mixture was subjected to vigorous vortex stirring for 30 seconds 4 times, with 30-second intervals, and at 4°C. After this, the soluble fraction was recovered by centrifuging the lysis mixture at 13,000 rpm for 15 minutes at 4°C. This sample was subjected to fractioning on a sucrose gradient according to a previously described protocol (Lombardo, E., Maraver, A., Castón, J. R., Rivera, J., Fernández-Arias, A., Serrano, A., Carrascosa, J. L. & Rodríguez, J. F. (1999). VP1, the putative RNA-dependent RNA polymerase of infectious bursal disease virus, forms complexes with the capsid protein VP3, leading to efficient encapsidation into virus-like particles. *Journal of Virology* 73, 6973-6983). The samples obtained after fractioning as well as a sample of the starting material were analyzed by means of sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) [Current Protocols in Molecular Biology] and immunodetection by Western blot (Figure 4A) using anti-pVP2 and anti-VP3 sera [Current Protocols in Molecular Biology]. As is shown in Figure 4A, the Western blot showed the presence of bands, with the predicted molecular mass corresponding to the pVP2 (48 kDa) and VP3-GFP (61 kDa) proteins, as well as other immunoreactive bands of a smaller size probably produced by proteolytic degradation both in the initial sample and in the different

fractions of the gradient. These results reliably showed the correct expression of both polypeptides in the *S. cerevisiae* culture transformed with the plasmid pESCURA/pVP2-VP3. Then, the different fractions of the gradient were analyzed by means of TEM as has been previously described (Lombardo, E., Maraver, A., Castón, J. R., Rivera, J., Fernández-Arias, A., Serrano, A., Carrascosa, J. L. & Rodríguez, J. F. (1999). VP1, the putative RNA-dependent RNA polymerase of infectious bursal disease virus, forms complexes with the capsid protein VP3, leading to efficient encapsidation into virus-like particles. *Journal of Virology* 73, 6973-6983). As is shown in Figure 4B, the TEM analysis of the fractions of the gradient showed the existence of IBDV CVLPs in the top fractions of the gradient. These CVLPs have a diameter of 65-70 nm and a polygonal contour that is indistinguishable from the IBDV CVLPs obtained in other expression systems (Figure 4C).

### EXAMPLE 3

#### Obtaining and characterizing the immunogenicity of IBDV CVLPs

As part of the development of new vaccination strategies, the possibility of using the strategy of producing chimeric IBDV VLPs (CVLPs) which contained heterologous amino acid sequences corresponding to other proteins or peptides involved in the induction of an immune response was analyzed. As a study model, the possibility of obtaining CVLPs which contained, as a heterologous polypeptide comprising a polypeptide of interest, the amino acid sequence corresponding to the CD8 epitope (E-CD8) of the malaria CS protein (*Plasmodium yoelii*), was approached. (Quantification of antigen specific CD8+ T cells using an ELISPOT assay. J Immunol Methods 181: 45-54; Zavala, F., Rodrigues, M., Rodriguez, D., Rodriguez, J. R., Nussenzweig, R. S. and Esteban, M. (2001). A striking property of recombinant poxviruses: efficient inducers of in vivo expansion of primed CD8(+) T cells. *Virology* 280: 155-159). This epitope is responsible for the CD8-specific cellular immune response induction against this pathogen (Oliveira-Ferreira J, Miyahira Y, Layton GT, Savage N, Esteban M, Rodriguez D, Rodriguez JR, Nussenzweig RS, Zavala F, Myahira Y. (2000). Immunogenicity of Ty-VLP bearing a CD8(+) T cell epitope of the CS protein of *P. yoelii*: enhanced memory response by boosting with recombinant vaccinia virus. *Vaccine* 18: 1863-1869). This response can be quantified by means of the ELISPOT technique (Miyahira Y, Murata K, Rodriguez D, Rodriguez JR, Esteban M, Rodrigues MM, Zavala F. (1995) Quantification of antigen specific CD8+ T cells using an ELISPOT assay. J Immunol Methods 181: 45-54) in splenocyte cultures from BALB/c mice.

For this purpose, the construction of the plasmid pFB/his-CD8-VP3 (SEQ ID NO: 13) was carried out following the cloning strategy described later. This vector was constructed by means of insertion of a 36 bearing portion DNA fragment, generated by means of hybridization of the synthetic oligonucleotides identified as CD8 A (SEQ ID NO: 11) and CD8 B (SEQ ID NO: 12), containing the encoding sequence of the CD8 epitope (SYVPSAEQI, see residues 29 to 37 of the SEQ ID NO: 13 and 14) of the malaria CS protein in the ORF encoding the his-VP3 protein integrated in the pFB/his-VP3 vector. The cloning was carried out by means of ligation of the DNA fragment generated by means of hybridization of the synthetic oligonucleotides CD8 A and B (SEQ ID NO: 11 and SEQ ID NO: 12) to the plasmid pFB/his-VP3 digested with the EheI restriction enzyme. This plasmid pFB/his-CD8-VP3 (SEQ ID NO: 13) contains and ORF encoding a fusion protein called his-CD8-VP3 containing the CD8 epitope inserted in the end corresponding to the N-terminal sequence of the his-VP3 protein ORF. The amino acid sequence of the his-CD8-VP3 fusion protein encoded by the nucleotide sequence contained in said plasmid pFB/his-CD8-VP3 is shown in SEQ ID NO: 14.

The plasmid pFB/his-CD8-VP3 was purified and used to generate the corresponding recombinant baculovirus (rBV), called FB/his-CD8-VP3, following the Bac-to-Bac technology according to the protocols described by the manufacturer (Invitrogen BV, Groningen, The Netherlands).

### 3.1 Producing CVLPs

H5 cell cultures were simultaneously infected with the recombinant baculoviruses FB/His-CD8-VP3 and FB/pVP2. The FB/ pVP2 rBV (see Example 1.1) expresses the region corresponding to the pVP2 protein (Met1-Ala 512) of the IBDV polyprotein. The cells were harvested at 48 hours post-infection (pi), and the corresponding extracts were subjected to the IBDV VLPs purification protocol by means of fractioning on linear sucrose gradients (Lombardo, E., Maraver, A., Castón, J. R., Rivera, J., Fernández-Arias, A., Serrano, A., Carrascosa, J. L. & Rodríguez, J. F. (1999). VP1, the putative RNA-dependent RNA polymerase of infectious bursal disease virus, forms complexes with the capsid protein VP3, leading to efficient encapsidation into virus-like particles. *Journal of Virology* 73: 6973-6983). Each one of the obtained fractions was viewed by means of transmission electron microscopy (TEM) and analyzed by means of SDS-PAGE and immunoblot using VP3 specific antibody. As is observed in Figure 5A, fraction 4 of the gradient contained abundant assemblies with an identical structure (polygonal perimeter and a diameter of 65-70 nm) as the IBDV VLPs

obtained by means of expression of the viral polyprotein. The biochemical characterization, by means of SDS-PAGE and Western blot (Figure 5B), showed that these CVLPs contain a protein, immunoreactive against the anti-VP3 serum, the molecular mass (33.5 kDa) of which is identical to the aforementioned one for the his-CD8-VP3 fusion protein (33.591 kDa). These results allow concluding that the coexpression of the pVP2 and his-CD8-VP3 genes in insect cells gives rise to the formation of chimeric VLPs (CVLPs) containing the his-CD8-VP3 fusion protein. These CVLPs are called CD8-CVLPs.

### 3.2 Immunogenicity analysis of CD8-CVLPs

For the purpose of determining the immunogenic capacity of the CD8-CVLPs two identical assays were carried out using two batches of CD8-CVLPs independently produced and purified. Four groups (I, II, III and IV) of three female eight-week old BalbC rats were used. The groups were formed randomly. The immunization strategy was similar to the one used previously in the characterization of other immunogens. This strategy is based on the use of a priming dose with the antigen under study, followed by a second booster dose, which amplifies the primary response, with the recombinant vaccinia virus VVpJRCS, which expresses the malaria CS protein. The induced immune response was determined by means of the detection of the antigen specific CD8<sup>+</sup> T cells according to their ability to produce IFN- $\gamma$ , by means of an ELISPOT assay (Miyahira Y, Murata K, Rodriguez D, Rodriguez JR, Esteban M, Rodrigues MM, Zavala F. (1995). Quantification of antigen specific CD8<sup>+</sup> T cells using an ELISPOT assay. J Immunol Methods 181: 45-54). In summary, 96-well plates with nitrocellulose (Millipore) bottoms were coated with 75  $\mu$ l/well of a solution containing 6  $\mu$ g/ml of the rat anti-murine IFN- $\gamma$  monoclonal antibody (R4-6A2, Pharmingen, San Diego, CA) resuspended in PBS. The plates were incubated overnight at room temperature. The wells were subsequently washed three times with RPMI medium, and were finally incubated with RPMI medium supplemented with 10% fetal calf serum (FCS) for one hour at 37°C 5% CO<sub>2</sub> atmosphere. On the other hand, the spleens of the immunized rats, maintained in RPMI medium supplemented with 10% FCS, were arranged on a sterile grid on a 60 movable member plate and were homogenized, the extract breaking up by means of its passing through needles of different gauges (21G->25G). The cells thus broken up were centrifuged for 5 minutes at 1,500 rpm at 4°C, and were washed twice with RPMI + 10% FCS medium. In order to lyse the erythrocytes of the samples, sterile 0.1 M NH<sub>4</sub>Cl (2 ml/spleen) was added and it was maintained at 4°C for 3-5 minutes, RPMI + 10% FCS was added and it was centrifuged.

Then, they were twice and it was finally resuspended in 1-2 ml RPMI + 10% FCS. The splenocyte viability count was carried out by means of trypan blue staining (4% in water, Sigma).

The professional antigen-presenting cells (APCs) used in this assay were P815. These cells were adjusted to a concentration of  $10^6$  cells/ml and were incubated with the synthetic peptide SYVPSAEQI (corresponding to the CD8 region of the malaria CS protein)  $10^{-6}$  M. After treatment with the peptide, the cells were washed and treated with mitomycin C (30  $\mu$ g/ml) (Sigma) for 15 minutes at 37°C and in CO<sub>2</sub> atmosphere. After subsequent washings, the antigen-presenting cells, to which 30 U/ml of murine interleukin 2 (IL-2) were added, were added at a concentration of  $10^5$  cells/well. 100  $\mu$ l/well of  $10^6$  splenocytes/ml and 1/4 and 1/16 dilutions were also added. The plates were incubated for 18 ours at 37°C in CO<sub>2</sub> atmosphere, they were washed 5 times with PBST and incubated with 2  $\mu$ g/ml of the biotinylated rat anti-IFN- $\gamma$  XMG1.2 monoclonal antibody (Pharmingen) diluted in PBST for 2 hours at room temperature. Then the plates were washed five times with PBST and a dilution of 1/800 avidin-peroxidase was added (0.5 mg/ml) (Sigma). After 1 hour of incubation at room temperature, it was washed 3 times with PBST and 2 times with PBS, finally adding the developer mixture with 1  $\mu$ g/ml of the DAB substrate (Sigma), resuspended in Tris-HCl, pH 7.5, 50 mM, containing 0.015% H<sub>2</sub>O<sub>2</sub>. The reaction was stopped by washing the plate with abundant water, and once dried, the spots were counted with the aid of a Leica MZ122 APO stereomicroscope and the QWIN Imaging System software (Leica, Cambridge, United kingdom).

Immunizations were carried out according to the immunization program described in the following table:

Group	1 <sup>st</sup> Immunization. Day 0	2 <sup>nd</sup> Immunization. Day 14
I	Not immunized	VVpJRPyCS
II	VVpJRPyCS	VVpJRPyCS
III	IBDV VLPs	VVpJRPyCS
IV	CD8-CVLPs	VVpJRPyCS

Immunizations with VVpJRCS were carried out intraperitoneally using  $10^7$  plaque forming units (pfu) per anima. Immunizations with VLPs, both non-chimeric IBDV VLPs and CD8-CVLPs, were carried out intraperitoneally with a dose of 50  $\mu$ g of antigen per animal. In all cases, the antigen preparations were diluted in phosphate-buffered saline (PBS).

28 days after the first immunization, the animals were sacrificed and there spleens were used to carry out the ELISPOT assays. These assays were carried out following the protocol described above. Virtually identical results were obtained in both assays. Figure 6 shows the results corresponding to the first assay. The obtained results demonstrate that  
5 when CD8-CVLPs are used as a priming dose followed by a booster dose with the VVpJRCS virus (group IV), a strong stimulation of the specific cellular immune response against the malaria CD8 epitope occurs. This stimulation is much greater (about 20 times greater) than that obtained after the immunization with one (group I) or two (group II) doses of VVpJRCS. The fact that a significant stimulation of the response against E-CD8 dose not  
10 occur in animals immunized with non-chimeric IBDV VLPs (group III), with regard to group I, which received a single dose of VVpJRCS, demonstrates that the response obtained in group IV is specifically induced by the E-CD8 present in the his-CD8-VP3 fusion protein forming an integral part of the CD8-CVLPs.



## CLAIMS

1. A chimeric empty capsid of the infectious bursal disease virus (IBDV), characterized in that it is constituted by assembly of (i) IBDV pVP2 proteins and (ii) fusion  
5 proteins comprising a region A constituted by the IBDV VP3 protein bound to a region B constituted by a heterologous polypeptide comprising a polypeptide of interest.

2. Capsid according to claim 1, wherein said region B is bound to the amino-terminal region of IBDV VP3, or alternatively to the carboxy-terminal region of IBDV VP3.

10 3. Capsid according to claim 1, wherein said polypeptide of interest is a polypeptide useful in vaccination, therapy or diagnosis.

15 4. Capsid according to claim 1, wherein said region B comprises a single polypeptide of interest.

5. Capsid according to claim 1, wherein said region B comprises two or more polypeptides of interest.

20 6. Capsid according to claim 1, wherein said fusion protein comprises a region A bound to a single region B.

25 7. Capsid according to claim 1, wherein said fusion protein comprises a region A bound to two regions B, equal or different, one of them bound to the amino-terminal region of VP3 present in region A, and the other one to the carboxy-terminal region of VP3 present in region A.

8. Capsid according to claim 7, wherein said regions B contain more than one polypeptides of interest equal to or different from one another.

30 9. Capsid according to claim 1, wherein said fusion protein further comprises, a linker polypeptide located between said regions A and B.

10. A nucleic acid, said nucleic acid having a nucleotide sequence which comprises the nucleotide sequence encoding for the fusion protein defined in anyone of claims 1 to 9.

11. A nucleic acid, said nucleic acid having a nucleotide sequence which comprises  
5 (i) a nucleotide sequence comprising the open reading frame corresponding to the IBDV VP3 protein and (ii) a nucleotide sequence comprising the open reading frame of one or more heterologous polypeptides comprising one or more polypeptides of interest.

12. Nucleic acid according to claim 11, further comprising (iii) a nucleotide sequence  
10 comprising the open reading frame corresponding to the IBDV pVP2 protein.

13. A gene construct comprising a nucleic acid according to claim 10 or 11.

14. A gene construct comprising a nucleic acid according to claim 12.  
15

15. An expression system selected from:

a) an expression system comprising a first gene construct according to claim 13, operatively bound to transcription, and optionally translation, control elements, and a second gene construct, operatively bound to transcription, and optionally translation, control  
20 elements; said second gene construct comprising a nucleotide sequence comprising the open reading frame corresponding to the IBDV pVP2 protein; and

b) an expression system comprising a gene construct according to claim 14, operatively bound to transcription, and optionally translation, control elements.

25 16. Expression system according to claim 15, said expression system being selected from plasmids, bacmids, yeast artificial chromosomes (YACs), bacteria artificial chromosomes (BACs), bacteriophage P1-based artificial chromosomes (PACs), cosmids, or viruses, which optionally contain a heterologous replication origin.

30 17. A host cell containing a nucleic acid according to anyone of claims 10 to 12, or a gene construct according to anyon of claims 13 or 14, or an expression system according to anyone of claims 15 or 16.

18. A host cell, said cell having been transformed, transfected or infected with an expression system according to any of claims 15 or 16.

19. Host cell according to claim 17 or 18, said cell being selected from a mammal  
5 cell, an avian cell, an insect cell and a yeast.

20. A process for the production of chimeric empty capsids of the infectious bursal  
disease virus (IBDV) according to anyone of claims 1 to 9, comprising culturing a host cell  
according to anyone of claims 17 to 19, and, if desired, recovering said chimeric empty  
10 IBDV capsids.

21. Process according to claim 20, wherein said host cell is an insect cell, comprising  
the steps of:

- 15           a)     preparing an expression system selected from (I) and (II), wherein:
- expression system (I) is constituted by a recombinant baculovirus  
containing a gene construct according to claim 14; and
  - 20           - expression system (II) is constituted by a first recombinant baculovirus  
containing a gene construct encoding for the IBDV pVP2 protein, and a  
second recombinant baculovirus containing a gene construct according to  
claim 13;
- 25           b)     infecting insect cells with said expression system prepared in step a);
- c)     culturing the infected insect cells obtained in step b) under conditions  
allowing the expression of recombinant proteins and their assembly to form  
chimeric empty IBDV capsids; and
- 30           d)     if desired, isolating and optionally purifying the chimeric empty IBDV  
capsids.

22. A process according to claim 20, wherein said host cell is a yeast, comprising the steps of:

- 5 a) preparing an expression system constituted by a plasmid containing a gene construct according to claim 14;
- b) transforming yeast cells with said expression system prepared in step a);
- 10 c) culturing the transformed yeasts obtained in step b) under conditions allowing the expression of recombinant proteins and their assembly to form chimeric empty IBDV capsids; and
- d) if desired, isolating and optionally purifying the chimeric empty IBDV capsids.

15 23. The use of a gene expression system according to anyone of claims 15 or 16 for producing chimeric empty IBDV capsids according to anyone of claims 1 to 9.

20 24. The use of chimeric empty capsids of the infectious bursal disease virus (IBDV) according to anyone of claims 1 to 9 in the manufacture of a medicament.

25 25. Use according to claim 24, wherein said medicament is a vaccine.

26. Use according to claim 24, wherein said medicament is a gene therapy vector.

27. A vaccine comprising a therapeutically effective amount of chimeric empty capsids of the infectious bursal disease virus (IBDV) according to anyone of claims 1 to 9, optionally together with one or more pharmaceutically acceptable adjuvants and/or vehicles.

28. A vaccine according to claim 27, useful to simultaneously protect animals or  
30 humans against infection caused by two or more disease-causing infectious agents.

29. A gene therapy vector comprising a chimeric empty capsid of the infectious bursal disease virus (IBDV) according to anyone of claims 1 to 9.

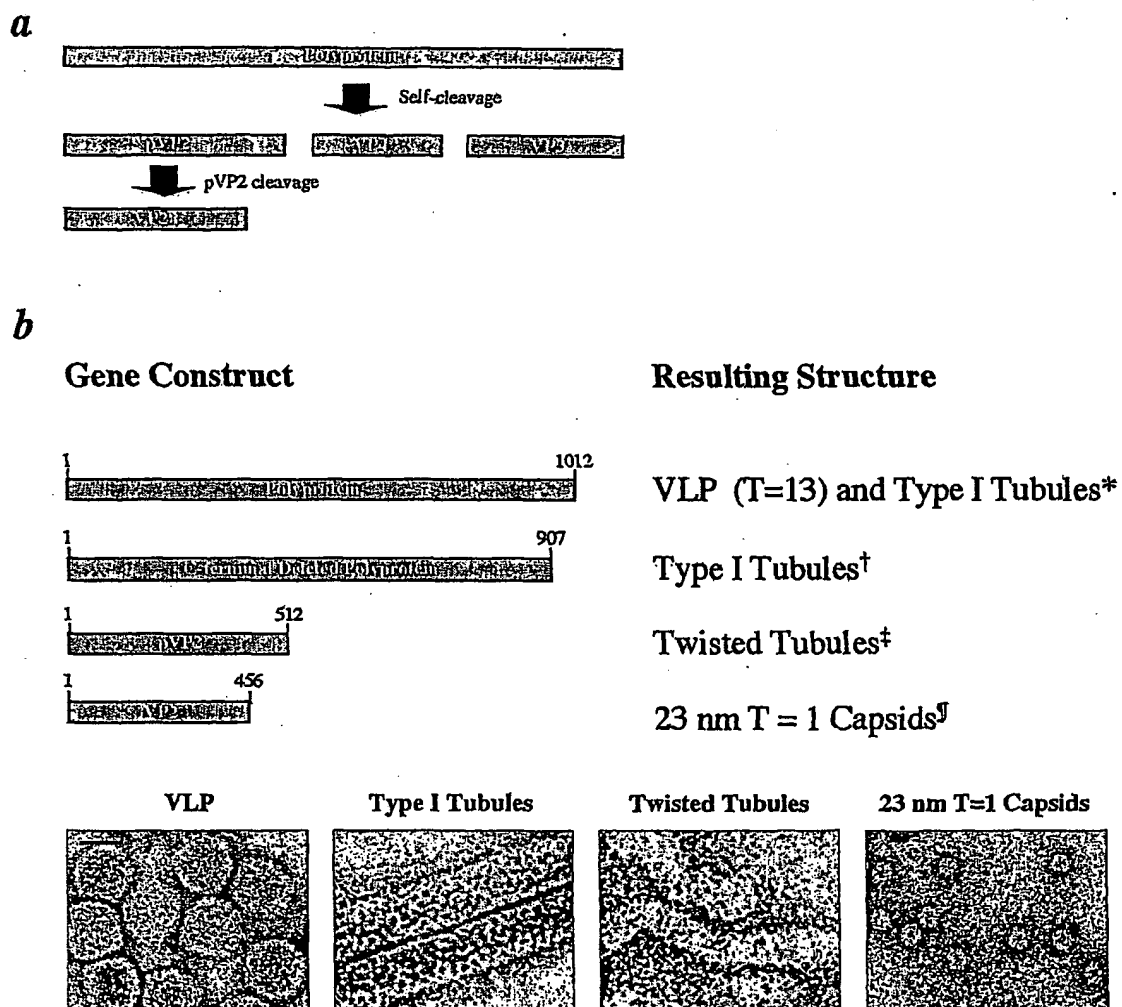


Fig. 1

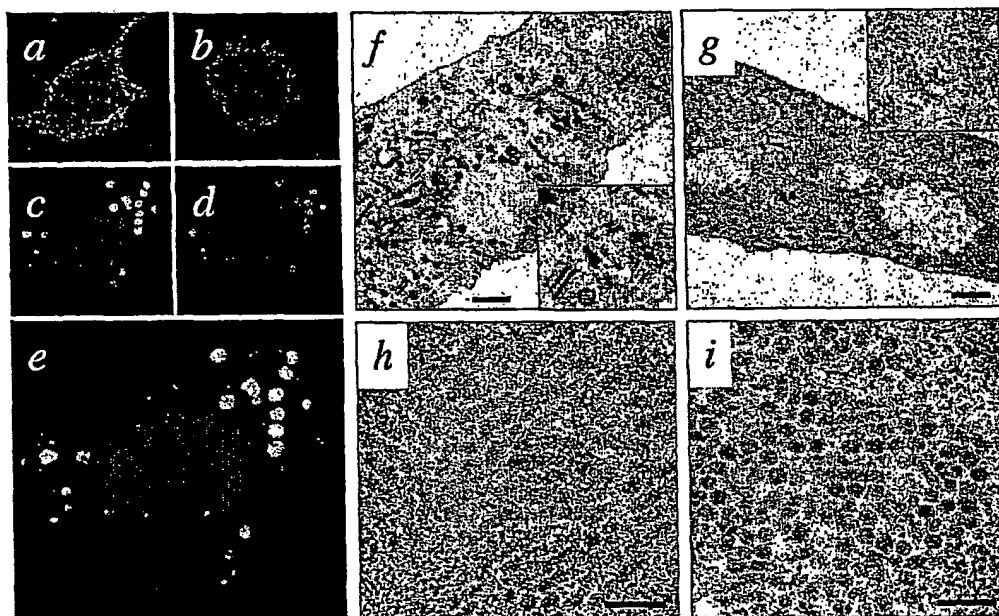


Fig. 2

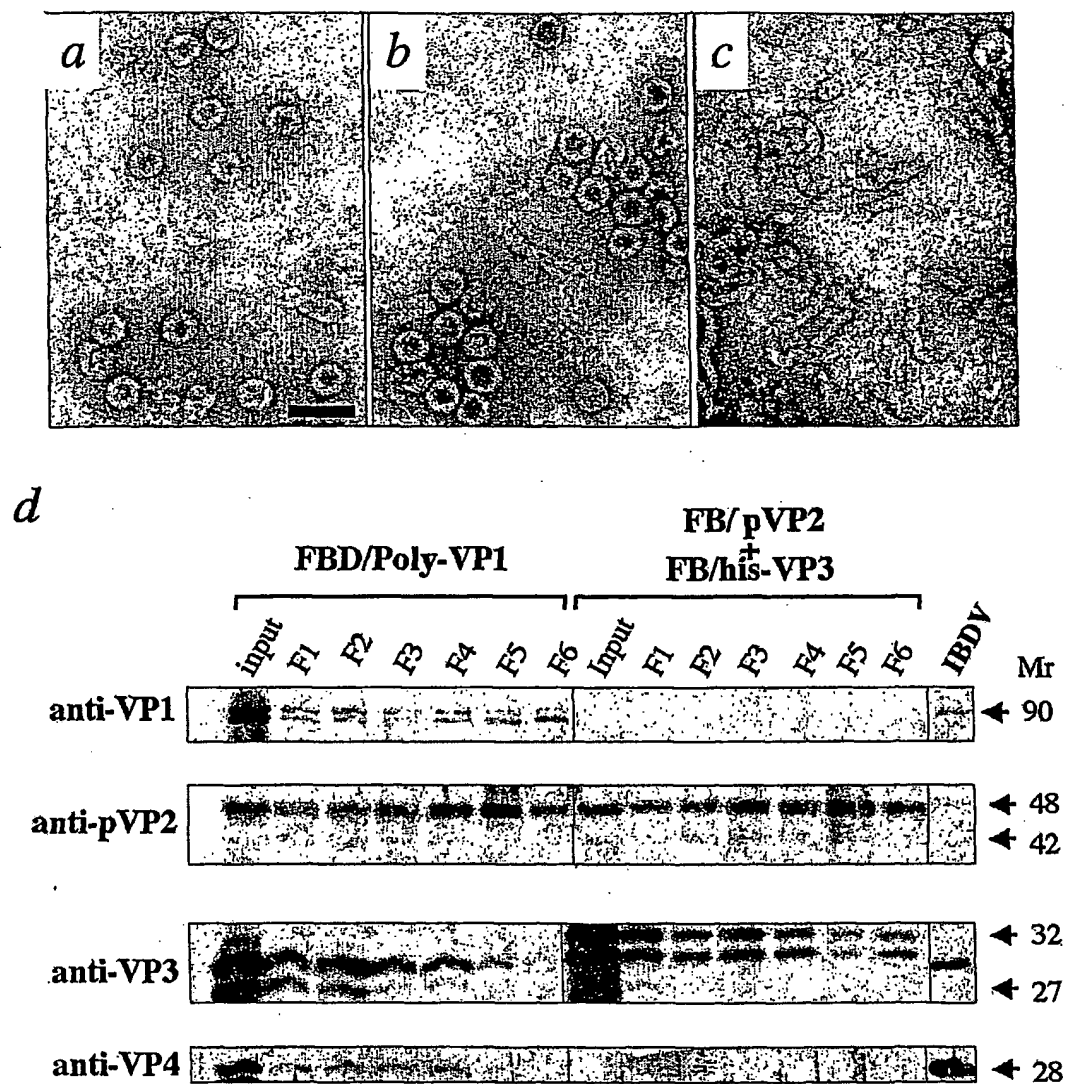


Fig. 3

4/6

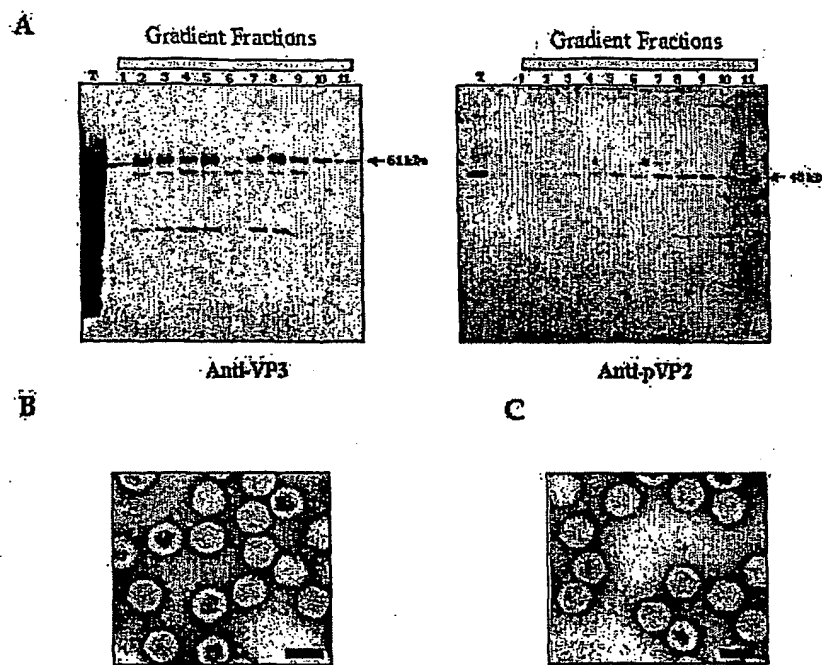
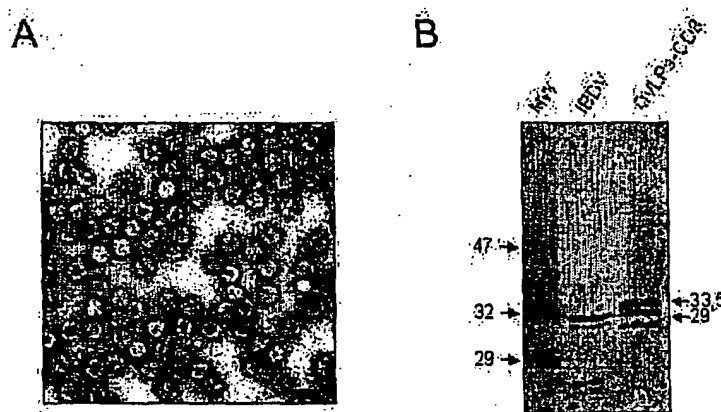


Fig. 4



**Fig. 5**

6/6

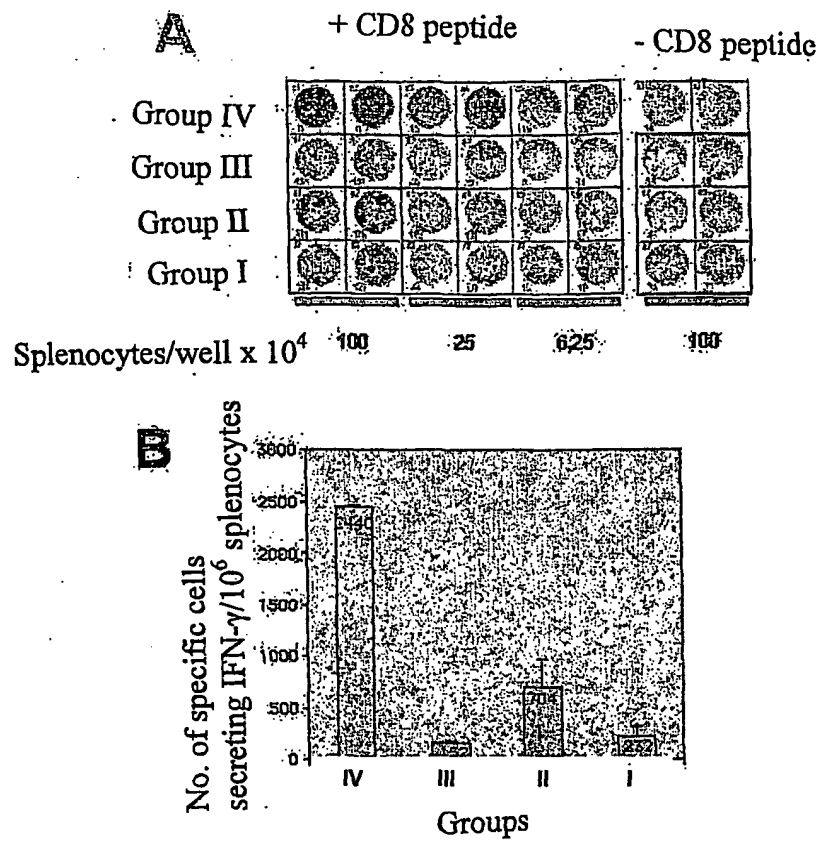


Fig. 6

## SEQUENCE LISTING

<110> CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS  
<110> BIONOSTRA, S.L.

<120> CHIMERIC EMPTY CAPSIDS OF THE INFECTIOUS BURSAL DISEASE VIRUS  
(IBDV), OBTAINMENT PROCESS AND APPLICATIONS

<130> P1391PC

<150> ES P200400120  
<151> 2004-01-21 (January 21, 2004)

<160> 14

<170> PatentIn version 3.1

<210> 1  
<211> 35  
<212> DNA  
<213> Artificial sequence

<220> Synthetic DNA  
<223> Oligo I primer

<400> 1  
gcgcagatct atgacaaacc tgtcagatca aaccc 35

<210> 2  
<211> 34  
<212> DNA  
<213> Artificial sequence

<220> Synthetic DNA  
<223> Oligo II primer

<400> 2  
gcgcaagctt aggcgagagt cagctgcctt atgc 34

<210> 3  
<211> 7595  
<212> DNA  
<213> Artificial sequence

<220>  
<223> Plasmid pFBD/pVP2-his-VP3

<220>  
<221> promoter  
<222> (157)..(285)  
<223> Promoter ppolh

<220>  
<221> CDS  
<222> (291)..(1289)  
<223> pVP2 ORF



ggg gtc acc gtc ctc agc tta ccc aca tca tat gat ctt ggg tat gtg Gly Val Thr Val Leu Ser Leu Pro Thr Ser Tyr Asp Leu Gly Tyr Val 165 170 175	824
agg ctt ggt gac ccc att ccc gca ata ggg ctt gac cca aaa atg gta Arg Leu Gly Asp Pro Ile Pro Ala Ile Gly Leu Asp Pro Lys Met Val 180 185 190	872
gcc aca tgt gac agc agt gac agg ccc aga gtc tac acc ata act gca Ala Thr Cys Asp Ser Ser Asp Arg Pro Arg Val Tyr Thr Ile Thr Ala 195 200 205 210	920
gcc gat gat tac caa ttc tca tca cag tac caa cca ggt ggg gta aca Ala Asp Asp Tyr Gln Phe Ser Ser Gln Tyr Gln Pro Gly Gly Val Thr 215 220 225	968
atc aca ctg ttc tca gcc aac att gat gcc atc aca agc ctc agc gtt Ile Thr Leu Phe Ser Ala Asn Ile Asp Ala Ile Thr Ser Leu Ser Val 230 235 240	1016
ggg gga gag ctc gtg ttt cga aca agc gtc cac ggc ctt gta ctg ggc Gly Gly Glu Leu Val Phe Arg Thr Ser Val His Gly Leu Val Leu Gly 245 250 255	1064
gcc acc atc tac ctc ata ggc ttt gat ggg aca acg gta atc acc agg Ala Thr Ile Tyr Leu Ile Gly Phe Asp Gly Thr Thr Val Ile Thr Arg 260 265 270	1112
gct gtg gcc gca aac aat ggg ctg acg acc ggc acc gac aac ctt atg Ala Val Ala Ala Asn Asn Gly Leu Thr Thr Gly Thr Asp Asn Leu Met 275 280 285 290	1160
cca ttc aat ctt gtg att cca aca aac gag ata acc cag cca atc aca Pro Phe Asn Leu Val Ile Pro Thr Asn Glu Ile Thr Gln Pro Ile Thr 295 300 305	1208
tcc atc aaa ctg gag ata gtg acc tcc aaa agt ggt ggt cag gca ggg Ser Ile Lys Leu Glu Ile Val Thr Ser Lys Ser Gly Gly Gln Ala Gly 310 315 320	1256
gat cag atg tca tgg tcg gca aga ggg agc cta gcagtgcga tccatgggtg Asp Gln Met Ser Trp Ser Ala Arg Gly Ser Leu 325 330	1309
caactatcca ggggccctcc gtcccgtcac gctagtggcc tacgaaagag tggcaacagg	1369
atccgtcggt acggtcgctg gggtagcaaa cttcgagctg atcccaaate ctgaactagc	1429
aaagaacctg gttacagaat acggccgatt tgaccagga gccatgaact acacaaaatt	1489
gatactgagt gagagggacc gtcttggcat caagaccgtc tggccaacaa gggagtacac	1549
tgactttcgt gaatacttca tggaggtggc cgacctcaac tctcccctga agattgcagg	1609
agcattcggc ttcaaagaca taatccgggc cataaggagg atagctgtgc cggtggtctc	1669
cacattgttc ccacctgccg ctcccctagc ccatgcaatt ggggaagggtg tagactacct	1729
gctgggcat gaggcccagg ccgcttcagg aactgctcga gccgcgtcag gaaaagcaag	1789

agctgcctca ggccgcataa ggcagctgac tctcgccataa gcttgctcgag aagtactaga 1849  
ggatcataat cagccatacc acatttgtag aggttttact tgctttaaaa aacctcccac 1909  
acctccccct gaacctgaaa cataaaatga atgcaattgt tgttggttaac ttgtttattg 1969  
cagcttataa tggttacaaa taaagcaata gcatcacaaa tttcacaaat aaagcatttt 2029  
tttcaactgca ttctagttgt ggtttggtcca aactcatcaa tgtatcttat catgtctgga 2089  
tctgatcact gcttgagcct aggagatccg aaccagataa gtgaaatcta gttccaaact 2149  
atthttgtcat ttttaatttt cgtatttagct tacgacgcta caccagttc ccatctatth 2209  
tgtcactctt ccttaaataa tcttaaaaa ctccatttcc acctctcca gttcccaact 2269  
atthttgtccg cccacagcgg ggcatttttc ttctgttat gtttttaate aaacatcctg 2329  
ccaactccat gtgacaaacc gtcatcttgc gctacttttt ctctgtcaca gaatgaaaat 2389  
ttttctgtca tctcttcgtt attaatgttt gtaattgact gaatatcaac gcttatttgc 2449  
agcctgaatg gcgaatggga cgcgcctgt agcggcgcat taagcgcggc gggtgtggtg 2509  
gttacgcgca gcgtgaccgc tacacttgcc agcgcctag cgcgcgtcc tttcgtttc 2569  
ttcccttctt ttctcgccac gttcgccggc tttcccgctc aagctctaaa tcgggggctc 2629  
cctttagggt tccgatttag tgctttacgg cactcgacc ccaaaaaact tgattagggt 2689  
gatggttcac gtagtgggcc atcgccctga tagacggttt ttcgcccttt gacgttggag 2749  
tccacgttct ttaatagtgg actcttggtc caaactggaa caaactcaa ccttatctcg 2809  
gtctattctt ttgatttata agggattttg cggatttcgg cctattgggt aaaaaatgag 2869  
ctgatttaac aaaaatttaa cgcgaatttt aacaaaatat taacgtttac aatttcaggt 2929  
ggcacttttc ggggaaatgt gcgcggaacc cctatttggt tatttttcta aatacattca 2989  
aatatgtatc cgctcatgag acaataacc tgataaatgc ttcaataata ttgaaaaagg 3049  
aagagtatga gtattcaaca tttcgtgtc gcccttattc ctttttttgc ggcattttgc 3109  
cttctgttt ttgctcacc agaaacgtg gtgaaagtaa aagatgctga agatcagttg 3169  
ggtgcacgag tgggttacat cgaactggat ctcaacagcg gtaagatcct tgagagtttt 3229  
cgccccgaag aacgttttcc aatgatgagc acttttaag ttctgctatg tggcgcggta 3289  
ttatcccgta ttgacgccg gcaagagcaa ctcggtcgcc gcatacacta ttctcagaat 3349  
gacttggttg agtactcacc agtcacagaa aagcatctta cggatggcat gacagtaaga 3409  
gaattatgca gtgctgcat aaccatgagt gataacactg cggccaactt acttctgaca 3469  
acgatcggag gaccgaagga gctaaccgct tttttgcaca acatggggga tcatgtaact 3529  
cgccttgatc gttgggaacc ggagctgaat gaagccatac caaacgacga gcgtgacacc 3589

acgatgcctg tagcaatggc aacaacgttg cgcaaactat taactggcga actacttact 3649  
ctagcttccc ggcaacaatt aatagactgg atggaggcgg ataaagttgc aggaccactt 3709  
ctgcgctcgg cccttcgggc tggctggttt attgctgata aatctggagc cggtgagcgt 3769  
gggtctcgg gtatcattgc agcactgggg ccagatggta agccctcccg tatcgtagtt 3829  
atctacacga cggggagtca ggcaactatg gatgaacgaa atagacagat cgctgagata 3889  
ggtgcctcac tgattaagca ttggtaactg tcagaccaag ttactcata tatacttttag 3949  
attgatttaa aacttcattt ttaattttaa aggatctagg tgaagatcct ttttgataat 4009  
ctcatgacca aaatccctta acgtgagttt tcgttccact gagcgtcaga ccccgtagaa 4069  
aagatcaaag gatcttcttg agatcctttt tttctgcgcg taatctgctg cttgcaaaca 4129  
aaaaaaccac cgctaccagc ggtggtttgt ttgccggatc aagagctacc aactcttttt 4189  
ccgaaggtaa ctggcttcag cagagcgagc ataccaaata ctgtccttct agtgtagcgg 4249  
tagttaggcc accacttcaa gaactctgta gcacgccta catacctcgc tctgctaate 4309  
ctgttaccag tggctgctgc cagtggcgat aagtcgtgtc ttaccgggtt ggactcaaga 4369  
cgatagttac cggataaggc gcagcggctg ggctgaacgg ggggttcgtg cacacagccc 4429  
agcttggagc gaacgaccta caccgaactg agatacctac agcgtgagca ttgagaaagc 4489  
gccacgcttc ccgaaggag aaaggcggac aggtatccgg taagcggcag ggtcggaaca 4549  
ggagagcgca cgaggagct tccaggggga aacgcctggg atctttatag tcctgtcggg 4609  
tttcgccacc tctgacttga gcgtcgattt ttgtgatgct cgtcaggggg gcggagccta 4669  
tggaaaaacg ccagcaacgc ggccttttta cggttcctgg ccttttgctg gccttttgct 4729  
cacatgttct ttctgcgtt atccctgat tctgtggata accgtattac cgcctttgag 4789  
tgagctgata ccgctcgccg cagccgaacg accgagcgca gcgagtcagt gagcgaggaa 4849  
gcggaagagc gcctgatgcg gtattttctc cttacgcate tgtgcggtat ttcacaccgc 4909  
agaccagccg cgtaacctgg caaaatcggg tacggttgag taataaatgg atgccctgcg 4969  
taagcgggtg tgggcggaca ataaagtctt aaactgaaca aaatagatct aaactatgac 5029  
aataaagtct taaactagac agaatagttg taaactgaaa tcagtcagat tatgctgtga 5089  
aaaagcatac tggacttttg ttatggctaa agcaaactct tcattttctg aagtgcaaata 5149  
tgcccgctcg attaaagagg ggcgtggcca agggcatggg aaagactata ttgcggcgct 5209  
tgtgacaatt taccgaacaa ctccgoggcc gggaagccga tctcggttg aacgaattgt 5269  
taggtggcgg tacttgggtc gatatacaag tgcatacatt cttcccgat gcccaacttt 5329  
gtatagagag cactgcggg atcgtcaccg taatctgctt gcacgtagat cacataagca 5389

ccaagcgcgt tggcctcatg cttgaggaga ttgatgagcg cgggtggcaat gccctgcctc 5449  
cgggtgctcgc cggagactgc gagatcatag atatagatct cactacgcgg ctgctcaaac 5509  
ctgggcagaa cgtaagccgc gagagcgcca acaaccgctt cttggtcgaa ggcagcaagc 5569  
gcgatgaatg tcttactacg gagcaagttc ccgaggtaat cggagtccgg ctgatgttgg 5629  
gagtaggttg ctacgtctcc gaactcacga ccgaaaagat caagagcagc ccgcatggat 5689  
ttgacttggt cagggccgag cctacatgtg cgaatgatgc ccatacttga gccacctaac 5749  
tttgttttag ggcgactgcc ctgctgcgta acatcggtgc tgctgcgtaa catcggttgc 5809  
gctccataac atcaaacatc gacccacggc gtaacgcgct tgctgcttgg atgcccgagg 5869  
catagactgt acaaaaaaac agtcataaca agccatgaaa accgccactg cgccgttacc 5929  
accgctgcgt tcggtcaagg ttctggacca gttgcgtgag cgcatacgtc acttgcatta 5989  
cagtttacga accgaacagg cttatgtcaa ctgggttcgt gccttcatcc gtttccacgg 6049  
tgtgcgtcac ccggcaacct tgggcagcag cgaagtcgag gcatttctgt cctggctggc 6109  
gaacgagcgc aaggtttcgg totccacgca tcgtcaggca ttggcggcct tgctgttctt 6169  
ctacggcaag gtgctgtgca cggatctgcc ctggcttcag gagatcggtg gacctcggcc 6229  
gtcgcggcgc ttgccggtgg tgctgacccc ggatgaagtg gttcgcaccc tcggttttct 6289  
ggaaggcgag catcgtttgt tcgcccagga ctctagctat agttctagt gttggcctac 6349  
gtacccgtag tggctatggc agggcttgcc gccccgacgt tggctgcgag ccctgggcct 6409  
tcacccgaac ttgggggttg ggggtgggaa aaggaagaaa cgcgggcgta ttggtccaa 6469  
tggggtctcg gtggggtatc gacagagtgc cagccctggg accgaacccc gcgtttatga 6529  
acaaacgacc caacaccogt gcgttttatt ctgtcttttt attgccgtca tagcgcgggt 6589  
tccttccggt attgtctcct tcogtgtttc agttagcctc ccccatctcc cggtagcgca 6649  
tgctctgaga ctgcaggctc tagattcgaa agcggccgcg actagtgagc tcgtcgacgt 6709  
aggcctttga attcoggatc ctcaactcaag gtctcatca gagacgggtc tgatccagcg 6769  
gccagccga ccagggggtc tctgtgttgg agcattgggt tttggcttgg gctttggtag 6829  
agcccgctg ggattgcgat gcttcatctc catcgagtc aagagcagat ctttcatctg 6889  
ttcttggttt gggccacgtc catggttgat ttcatagact ttggcaactt cgtctatgaa 6949  
agcttgggggt ggctctgcct gtctggagc ccgtagatc gacgtagctg cccttaggat 7009  
ttgttcttct gatgccaacc ggctcttctc tgcatgcacg tagtctagat agtcctcggt 7069  
tgggtccggt atttctcggt tgttctgcca gtactttacc tggcctgggc ttggccctcg 7129  
gtgccattg agtgetaccc attctgggtg tgcaaagtag atgcccatgg totccatctt 7189



ctttgagatc cgtgtgtctt ttccctctg tgcttctct ggtgtggggc cccgagcctc 7249  
 cactccgtag cctgctgtcc cgtacttggc cctttgcgac ttgctgcctg cttgtggtgc 7309  
 gtttgcaaga aaatttgcga tccgatgggc gttcgggtcg ctgagtgcga agttggccat 7369  
 gtcagtcaca atcccattct cttccagcca catgaacaca ctgagtgcag attggaatag 7429  
 tgggtccacg ttggctgctg cttccattgc tctgacggca ctctcgagtt cgggggtctc 7489  
 tttgaactct gatgcagcca tggcgccctg aaaatacagg ttttcggtcg ttgggatatc 7549  
 gtaatcgtga tggtagtggt gatggtagta cgacatggtt tcggac 7595

<210> 4

<211> 333

<212> PRT

<213> Artificial sequence

<220>

<223> pVP2-his-VP3 protein

<400> 4

Met Thr Asn Leu Ser Asp Gln Thr Gln Gln Ile Val Pro Phe Ile Arg  
1 5 10 15

Ser Leu Leu Met Pro Thr Thr Gly Pro Ala Ser Ile Pro Asp Asp Thr  
20 25 30

Leu Glu Lys His Thr Leu Arg Ser Glu Thr Ser Thr Tyr Asn Leu Thr  
35 40 45

Val Gly Asp Thr Gly Ser Gly Leu Ile Val Phe Phe Pro Gly Phe Pro  
50 55 60

Gly Ser Ile Val Gly Ala His Tyr Thr Leu Gln Gly Asn Gly Asn Tyr  
65 70 75 80

Lys Phe Asp Gln Met Leu Leu Thr Ala Gln Asn Leu Pro Ala Ser Tyr  
85 90 95

Asn Tyr Cys Arg Leu Val Ser Arg Ser Leu Thr Val Arg Ser Ser Thr  
100 105 110

Leu Pro Gly Gly Val Tyr Ala Leu Asn Gly Thr Ile Asn Ala Val Thr  
115 120 125

Phe Gln Gly Ser Leu Ser Glu Leu Thr Asp Val Ser Tyr Asn Gly Leu  
130 135 140

Met Ser Ala Thr Ala Asn Ile Asn Asp Lys Ile Gly Asn Val Leu Val  
145 150 155 160

Gly Glu Gly Val Thr Val Leu Ser Leu Pro Thr Ser Tyr Asp Leu Gly  
165 170 175

Tyr Val Arg Leu Gly Asp Pro Ile Pro Ala Ile Gly Leu Asp Pro Lys  
180 185 190

Met Val Ala Thr Cys Asp Ser Ser Asp Arg Pro Arg Val Tyr Thr Ile  
 195 200 205

Thr Ala Ala Asp Asp Tyr Gln Phe Ser Ser Gln Tyr Gln Pro Gly Gly  
 210 215 220

Val Thr Ile Thr Leu Phe Ser Ala Asn Ile Asp Ala Ile Thr Ser Leu  
 225 230 235 240

Ser Val Gly Gly Glu Leu Val Phe Arg Thr Ser Val His Gly Leu Val  
 245 250 255

Leu Gly Ala Thr Ile Tyr Leu Ile Gly Phe Asp Gly Thr Thr Val Ile  
 260 265 270

Thr Arg Ala Val Ala Ala Asn Asn Gly Leu Thr Thr Gly Thr Asp Asn  
 275 280 285

Leu Met Pro Phe Asn Leu Val Ile Pro Thr Asn Glu Ile Thr Gln Pro  
 290 295 300

Ile Thr Ser Ile Lys Leu Glu Ile Val Thr Ser Lys Ser Gly Gly Gln  
 305 310 315 320

Ala Gly Asp Gln Met Ser Trp Ser Ala Arg Gly Ser Leu  
 325 330

<210> 5  
 <211> 35  
 <212> DNA  
 <213> Artificial sequence

<220> Synthetic DNA  
 <223> Oligo III primer

<400> 5  
 gcgcagatct atgacaaacc tgtcagatca aaccc

35

<210> 6  
 <211> 34  
 <212> DNA  
 <213> Artificial sequence

<220> Synthetic DNA  
 <223> Oligo IV primer

<400> 6  
 gcgcaagctt aggcgagagt cagctgcctt atgc

34

<210> 7  
 <211> 9600  
 <212> DNA  
 <213> Artificial sequence

<220>  
 <223> Plasmid pESCURA/pVP2-VP3-GFP

<220>  
 <221> promoter  
 <222> (5649)..(5859)  
 <223> Promoter 1 (pVP2)

<220>  
 <221> promoter  
 <222> (7402)..(8080)  
 <223> Promoter 2 (VP3-GFP)

<220>  
 <221> CDS  
 <222> (8086)..(9597)  
 <223> VP3-GFP ORF

<400> 7  
 ggccgcacta gtatcgatgg attacaagga tgacgacgat aagatctgag ctcttaatta 60  
 acaattcttc gccagagggtt tgggtcaagtc tccaatcaag gttgtcggct tgtctacctt 120  
 gccagaaatt tacgaaaaga tggaaaaggg tcaaatcggt ggtagatacg ttgttgacac 180  
 ttctaaataa gcgaatttct tatgatttat gatttttatt attaaataag ttataaaaaa 240  
 aataagtgtat taaaaatttt aaagtgactc ttaggtttta aaacgaaaat tcttattctt 300  
 gagtaactct ttctgttagg tcagggttgct ttctcaggta tagcatgagg tcgctccaat 360  
 tcagctgcat taatgaatcg gccaacgcgc ggggagaggc ggtttgcgta ttgggcgctc 420  
 ttccgcttcc tcgctcactg actcgctgcg ctcggtcggt cggctgcggc gagcgggtatc 480  
 agctcactca aaggcggtaa tacggttatc cacagaatca ggggataacg caggaaagaa 540  
 catgtgagca aaaggccagc aaaaggccag gaaccgtaaa aaggccgcgt tgctggcggtt 600  
 tttccatagg ctccgcccc ctgacgagca tcacaaaaat cgacgctcaa gtcagagggtg 660  
 gcgaaacccg acaggactat aaagatacca ggcgtttccc cctggaagct ccctcgtgcg 720  
 ctctcctgtt ccgaccctgc cgcttaccgg atacctgtcc gcctttctcc ctccgggaag 780  
 cgtggcgctt tctcatagct cacgctgtag gtatctcagt tcggtgtagg tcgttcgctc 840  
 caagctgggc tgtgtgcacg aacccccgt tcagcccgac cgctgcgcct tatccggtaa 900  
 ctatcgtctt gagtccaacc cggtaagaca cgacttatcg cactggcag cagccactgg 960  
 taacaggatt agcagagcga ggtatgtagg cgggtgctaca gagttcttga agtgggtggc 1020  
 taactacggc tacactagaa ggacagtatt tggatctgc gctctgctga agccagttac 1080  
 ctccggaaaa agagtggta gctcttgatc cggcaaaaa accaccgctg gtagcgggtg 1140  
 tttttttgtt tgcaagcagc agattacgcg cagaaaaaaa ggatctcaag aagatccttt 1200  
 gatcttttct acggggtctg acgctcagtg gaacgaaaac tcacgttaag ggattttggt 1260

catgagatta tcaaaaagga tcttcaccta gatcctttta aattaaaaat gaagttttta 1320  
atcaatctaa agtatatatg agtaaaacttg gtctgacagt taccaatgct taatcagtga 1380  
ggcacctatc tcagcgatct gtctatttcg ttcattccata gttgcctgac tccccgtcgt 1440  
gtagataact acgatacggg agggccttacc atctggcccc agtgctgcaa tgataccgcg 1500  
agaccacgc tcaccggctc cagattttatc agcaataaac cagccagccg gaagggccga 1560  
gcgcagaagt ggtcctgcaa ctttatccgc ctccatccag tctattaatt gttgcggga 1620  
agctagagta agtagttcgc cagttaatag tttgcgcaac gttgttgcca ttgctacagg 1680  
catcgtggtg tcacgctcgt cgtttggtat ggcttcattc agtcocggtt cccaacgato 1740  
aaggcgagtt acatgatccc ccatgttgtg caaaaaagcg gttagctcct tcggtcctcc 1800  
gatcgttgtc agaagtaagt tggccgcagt gttatcactc atggttatgg cagcactgca 1860  
taattctctt actgtcatgc catccgtaag atgcttttct gtgactgggtg agtactcaac 1920  
caagtcattc tgagaatagt gtatgcggcg accgagttgc tottgcccgg cgtcaatacg 1980  
ggataatacc gcgccacata gcagaacttt aaaagtgtc atcattggaa aacgttcttc 2040  
ggggcgaaaa ctctcaagga tcttaccgct gttgagatcc agttcgatgt aaccactcg 2100  
tgcaccaaac tgatcttcag catcttttac tttcaccagc gtttctgggt gagcaaaaac 2160  
aggaaggcaa aatgccgcaa aaaagggaaat aaggcgaca cggaaatgtt gaatactcat 2220  
actcttctt tttcaatatt attgaagcat ttatcagggg tattgtctca tgagcggata 2280  
catatttgaa tgtattttaga aaaataaaca aataggggtt ccgcgcacat tccccgaaa 2340  
agtgccacct gaacgaagca tctgtgcttc attttgtaga acaaaaatgc aacgcgagag 2400  
cgctaatttt tcaaacaaag aatctgagct gcatttttac agaacagaaa tgcaacgcga 2460  
aagcgctatt ttaccaacga agaattctgtg cttcattttt gtaaaacaaa aatgcaacgc 2520  
gagagcgcta atttttcaaa caaagaatct gagctgcatt tttacagaac agaaatgcaa 2580  
cgcgagagcg ctattttacc aacaaagaat ctatacttct tttttgttct acaaaaatgc 2640  
atcccgagag cgctattttt ctaacaaagc atottagatt actttttttc tcctttgtgc 2700  
gctctataat gcagtctctt gataactttt tgcaactgtag gtccgttaag gttagaagaa 2760  
ggctactttg gtgtctatatt tctcttccat aaaaaagcc tgactccact tcccgcgttt 2820  
actgattact agcgaagctg cgggtgcatt ttttcaagat aaaggcatcc ccgattatat 2880  
tctataccga tgtggattgc gcatactttg tgaacagaaa gtgatagcgt tgatgattct 2940  
tcattgggtc gaaaattatg aacggtttct tctattttgt ctctatatac tacgtatagg 3000  
aaatgtttac attttcgtat tgttttcgat tcaactctatg aatagttctt actacaattt 3060

ttttgtctaa agagtaatac tagagataaa cataaaaaat gtagagggtcg agtttagatg 3120  
caagttcaag gagcgaaagg tggatgggta gggtatatag ggatatagca cagagatata 3180  
tagcaaagag atacttttga gcaatgtttg tggaagcggg attogcaata ttttagtagc 3240  
tcgttacagt ccggtgcgtt tttggttttt tgaaagtgcg tcttcagagc gcttttggtt 3300  
ttcaaaagcg ctctgaagtt cctatacttt ctagagaata ggaacttcgg aataggaact 3360  
tcaaagcgtt tccgaaaacg agcgcttcgg aaaatgcaac gcgagctgcg cacatacagc 3420  
tcaactgttca cgtcgcacct atatctgcgt gttgcctgta tatatatata catgagaaga 3480  
acggcatagt gcgtgtttat gcttaaagtc gtaactatat gcgtctatct atgtaggatg 3540  
aaaggtagtc tagtacctcc tgtgatatta tcccatcca tgcgggggtat cgtatgcttc 3600  
cttcagcact acccttttagc tgttctatat gctgccactc ctcaattgga ttagtctcat 3660  
ccttcaatgc tatcatttcc tttgatattg gatcatacta agaaaccatt attatcatga 3720  
cattaaccta taaaaatagg cgtatcaoga ggccttttcg tctcgcgctt ttcgggtgatg 3780  
acggtgaaaa cctctgacac atgcagctcc cggagacggg cacagcttgt ctgtaagcgg 3840  
atgccgggag cagacaagcc cgtcagggcg cgtcagcggg tgttggcggg tgtcggggct 3900  
ggcttaacta tgcggcatca gagcagattg tactgagagt gcaccatacc acagcttttc 3960  
aattcaattc atcatttttt ttttattctt ttttttgatt tcggtttctt tgaaattttt 4020  
ttgattcggg aatctccgaa cagaaggaag aacgaaggaa ggagcacaga cttagattgg 4080  
tatatatacg catatgtagt gttgaagaaa catgaaattg ccagttatc ttaaccaac 4140  
tgcacagaac aaaaacctgc aggaacgaa gataaatcat gtcgaaagct acatataagg 4200  
aacgtgctgc tactcatcct agtcctgttg ctgccaaagt atttaatatc atgcacgaaa 4260  
agcaaacaaa cttgtgtgct tcattggatg ttcgtaccac caaggaatta ctggagttag 4320  
ttgaagcatt aggtcccaa atttgtttac taaaaacaca tgtggatata ttgaactgatt 4380  
tttccatgga gggcacagtt aagccgctaa aggcattatc cgccaagtac aattttttac 4440  
tcttcgaaga cagaaaattt gctgacattg gtaatacagt caaattgcag tactctgcgg 4500  
gtgtatacag aatagcagaa tgggcagaca ttacgaatgc acacggtgtg gtgggcccag 4560  
gtattgttag cggtttgaag caggcggcag aagaagtaac aaaggaacct agaggccttt 4620  
tgatgttagc agaattgtca tgcaagggt ccctatctac tggagaatat actaagggt 4680  
ctgttgacat tgcaagagc gacaaagatt ttgttatcgg ctttattgct caaagagaca 4740  
tgggtggaag agatgaagg tactgattgg tgattatgac acccggtgtg ggttttagatg 4800  
acaagggaga cgcattgggt caacagtata gaaccgtgga tgatgtggtc tctacaggat 4860

ctgacattat tattgttggga agaggactat ttgcaaaggg aagggatgct aaggtagagg 4920  
gtgaacgtta cagaaaagca ggctgggaag catatttgag aagatgcggc cagcaaaact 4980  
aaaaaactgt attataagta aatgcatgta tactaaactc acaaattaga gottcaattt 5040  
aattatatca gttattaccc tatgcggtgt gaaataccgc acagatgcgt aaggagaaaa 5100  
taccgcatca ggaaattgta aacgttaata ttttgttaaa attcgcgtta aatttttgtt 5160  
aatcagctc attttttaac caataggccg aaatcggcaa aatcccttat aaatcaaaag 5220  
aatagaccga gataggggtg agtgttgttc cagtttgga caagagtcca ctattaaaga 5280  
acgtggactc caacgtcaaa gggcgaaaaa ccgtctatca gggcgatggc ccactacgtg 5340  
aaccatcacc ctaatcaagt tttttggggt cgaggtgccg taaagcacta aatcggaacc 5400  
ctaaagggag ccccgattt agagcttgac ggggaaagcc ggcgaacgtg gcgagaaagg 5460  
aagggaagaa agcgaaagga gcgggcgcta gggcgctggc aagtgtagcg gtcacgctgc 5520  
gcgtaaccac cacaccgccc gcgcttaatg cgccgctaca gggcgcgctc cgccattcgc 5580  
cattcaggct gcgcaactgt tgggaagggc gatcggtgcg ggccctctcg ctattacgcc 5640  
agctggatct tcgagcgtcc caaaaccttc tcaagcaagg ttttcagtat aatgttacat 5700  
gcgtacacgc gtctgtacag aaaaaaaga aaaatttgaa atataaataa cgttcttaat 5760  
actaacataa ctataaaaaa ataaataggg acctagactt caggttgtct aactccttcc 5820  
ttttcggtta gagcggatct tagctagccg cggtaccaag cttaggcgag agtcagctgc 5880  
cttatgcggc ctgaggcagc tcttgctttt cctgacgcgg ctcgagcagt tcctgaagcg 5940  
gcctgggcct catcgcccag caggtagtct acaccttccc caattgcatg ggctagggga 6000  
gcggcaggtg ggaacaatgt ggagaccacc ggcacagcta tcctccttat ggcccggatt 6060  
atgtctttga agccgaatgc tcctgcaatc ttcaggggag agttgaggtc ggccacctcc 6120  
atgaagtatt caogaaagtc agtgtaactc cttgttggcc agacggtctt gatgccaaga 6180  
cggtcctct cactcagtat caattttgtg tagttcatgg ctctggggtc aaatcggccg 6240  
tattctgtaa ccaggttctt tgctagttca ggatttggga tcagctcgaa gttgctcacc 6300  
ccagcgaccg taacgacgga tcctgttgcc actctttcgt aggccactag cgtgacggga 6360  
cggagggccc ctggatagtt gccaccatgg atcgtcactg ctaggctccc tcttgccgac 6420  
catgacatct gatccctgc ctgaccacca cttttggagg tcactatctc cagtttgatg 6480  
gatgtgattg gctgggttat ctcgtttgtt ggaatcacia gattgaatgg cataaggttg 6540  
tcggtgccgg tcgtcagccc attgtttgcg gccacagccc tggtgattac cgttgctcca 6600  
tcaaagccta tgaggtagat ggtggcgccc agtacaaggc cgtggacgct tgttcgaaac 6660

acgagctctc ccccaacgct gaggcttggt atggcatcaa tgttggctga gaacagtggtg 6720  
 attgttacct cacctggttg gtactgtgat gagaattggt aatcatcggc tgcagttatg 6780  
 gtgtagactc tgggcctgtc actgctgtca catgtggcta ccatttttgg gtcaagccct 6840  
 attgcgggaa tggggtcacc aagcctcaca tacccaagat catatgatgt gggtaagctg 6900  
 aggacggtga ccccttcccc tactaggacg ttcccaattt tgcgttgat gttggctggt 6960  
 gcagacatca acccattgta gctaacatct gtcagttcac tcaggcttcc ttggaaggtc 7020  
 acggcggtta tgggtgccgt tagtgcataa acgccaccag gaagtgtgct tgacctcact 7080  
 gtgagactcc gactcactag cctgcagtag ttgtaactgg ccggtagggt ctgggcagtc 7140  
 aggagcatct gatcgaactt gtagttccca ttgccctgca gtgtgtagtg agcaccaca 7200  
 attgagccag ggaatccagg gaaaaagaca attagccctg accctgtgtc cccacagtc 7260  
 aaattgtagg tcgaggtctc tgacctgaga gtgtgcttct ccagggtgtc gtccggaatg 7320  
 gacgccggtc cggttgttg catcagaagg ctccgtatga acggaacaat ctgctgggtt 7380  
 tgatctgaca ggtttgtcat agatccgggg ttttttctcc ttgacgttaa agtatagagg 7440  
 tatattaaca attttttgtt gatactttta ttacatttga ataagaagta atacaaaccg 7500  
 aaaatgttga aagtattagt taaagtgggt atgcagtttt tgcatttata tatctgttaa 7560  
 tagatcaaaa atcatcgctt cgctgattaa ttaccccaga aataaggcta aaaaactaat 7620  
 cgcattatca tcctatgggt gttaatttga ttcgttcatt tgaaggtttg tggggccagg 7680  
 ttactgccaa ttttctctc tcataaccat aaaagctagt attgtagaat ctttattgtt 7740  
 cggagcagtg cggcgcgagg cacatctgcg tttcaggaac gcgaccggtg aagacgagga 7800  
 cgcacggagg agagtcttcc ttcggagggc tgtcaccgc tcggcggctt ctaatccgta 7860  
 cttcaatata gcaatgagca gttaagcgta ttactgaaag ttcaaagag aaggtttttt 7920  
 taggctaaga taatggggct ctttacattt ccacaacata taagtaagat tagatatgga 7980  
 tatgtatatg gatatgtata tgggtgtaat gccatgtaat atgattatta aacttctttg 8040  
 cgtccatcca aaaaaaagt aagaattttt gaaaattcga attcg atg gct gca tca 8097  
 Met Ala Ala Ser  
 1  
 gag ttc aaa gag acc ccc gaa ctg gag agt gcc gtc aga gca atg gaa 8145  
 Glu Phe Lys Glu Thr Pro Glu Leu Glu Ser Ala Val Arg Ala Met Glu  
 5 10 15 20  
 gca gca gcc aac gtg gac cca cta ttc caa tct gca ctg agt gtg ttc 8193  
 Ala Ala Ala Asn Val Asp Pro Leu Phe Gln Ser Ala Leu Ser Val Phe  
 25 30 35  
 atg tgg ctg gaa gag aat ggg att gtg act gac atg gcc aac ttc gca 8241

Met Trp Leu Glu Glu Asn Gly Ile Val Thr Asp Met Ala Asn Phe Ala	
40 45 50	
ctc agc gac ccg aac gcc cat cgg atg cga aat ttt ctt gca aac gca	8289
Leu Ser Asp Pro Asn Ala His Arg Met Arg Asn Phe Leu Ala Asn Ala	
55 60 65	
cca caa gca ggc agc aag tcg caa agg gcc aag tac ggg aca gca ggc	8337
Pro Gln Ala Gly Ser Lys Ser Gln Arg Ala Lys Tyr Gly Thr Ala Gly	
70 75 80	
tac gga gtg gag gct cgg ggc ccc aca cca gag gaa gca cag agg gaa	8385
Tyr Gly Val Glu Ala Arg Gly Pro Thr Pro Glu Glu Ala Gln Arg Glu	
85 90 95 100	
aaa gac aca cgg atc tca aag aag atg gag acc atg ggc atc tac ttt	8433
Lys Asp Thr Arg Ile Ser Lys Lys Met Glu Thr Met Gly Ile Tyr Phe	
105 110 115	
gca aca cca gaa tgg gta gca ctc aat ggg cac cga ggg cca agc cca	8481
Ala Thr Pro Glu Trp Val Ala Leu Asn Gly His Arg Gly Pro Ser Pro	
120 125 130	
ggc cag gta aag tac tgg cag aac aaa cga gaa ata ccg gac cca aac	8529
Gly Gln Val Lys Tyr Trp Gln Asn Lys Arg Glu Ile Pro Asp Pro Asn	
135 140 145	
gag gac tat cta gac tac gtg cat gca gag aag agc cgg ttg gca tca	8577
Glu Asp Tyr Leu Asp Tyr Val His Ala Glu Lys Ser Arg Leu Ala Ser	
150 155 160	
gaa gaa caa atc cta agg gca gct acg tcg atc tac ggg gct cca gga	8625
Glu Glu Gln Ile Leu Arg Ala Ala Thr Ser Ile Tyr Gly Ala Pro Gly	
165 170 175 180	
cag gca gag cca ccc caa gct ttc ata gac gaa gtt gcc aaa gtc tat	8673
Gln Ala Glu Pro Pro Gln Ala Phe Ile Asp Glu Val Ala Lys Val Tyr	
185 190 195	
gaa atc aac cat gga cgt ggc cca aac caa gaa cag atg aaa gat ctg	8721
Glu Ile Asn His Gly Arg Gly Pro Asn Gln Glu Gln Met Lys Asp Leu	
200 205 210	
ctc ttg act gcg atg gag atg aag cat cgc aat ccc agg cgg gct cta	8769
Leu Leu Thr Ala Met Glu Met Lys His Arg Asn Pro Arg Arg Ala Leu	
215 220 225	
cca aag ccc aag cca aaa ccc aat gct cca aca cag aga ccc cct ggt	8817
Pro Lys Pro Lys Pro Lys Pro Asn Ala Pro Thr Gln Arg Pro Pro Gly	
230 235 240	
cgg ctg ggc cgc tgg atc agg acc gtc tct gat gag gac ctt gag gga	8865
Arg Leu Gly Arg Trp Ile Arg Thr Val Ser Asp Glu Asp Leu Glu Gly	
245 250 255 260	
tcc atc gcc acc atg gtg agc aag ggc gag gag ctg ttc acc ggg gtg	8913
Ser Ile Ala Thr Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val	
265 270 275	
gtg ccc atc ctg gtc gag ctg gac ggc gac gta aac ggc cac aag ttc	8961





<210> 8  
 <211> 503  
 <212> PRT  
 <213> Artificial sequence

<220>

<223> pVP2-VP3-GFP protein

<400> 8

Met	Ala	Ala	Ser	Glu	Phe	Lys	Glu	Thr	Pro	Glu	Leu	Glu	Ser	Ala	Val	1	5	10	15
Arg	Ala	Met	Glu	Ala	Ala	Ala	Asn	Val	Asp	Pro	Leu	Phe	Gln	Ser	Ala	20	25	30	
Leu	Ser	Val	Phe	Met	Trp	Leu	Glu	Glu	Asn	Gly	Ile	Val	Thr	Asp	Met	35	40	45	
Ala	Asn	Phe	Ala	Leu	Ser	Asp	Pro	Asn	Ala	His	Arg	Met	Arg	Asn	Phe	50	55	60	
Leu	Ala	Asn	Ala	Pro	Gln	Ala	Gly	Ser	Lys	Ser	Gln	Arg	Ala	Lys	Tyr	65	70	75	80
Gly	Thr	Ala	Gly	Tyr	Gly	Val	Glu	Ala	Arg	Gly	Pro	Thr	Pro	Glu	Glu	85	90	95	
Ala	Gln	Arg	Glu	Lys	Asp	Thr	Arg	Ile	Ser	Lys	Lys	Met	Glu	Thr	Met	100	105	110	
Gly	Ile	Tyr	Phe	Ala	Thr	Pro	Glu	Trp	Val	Ala	Leu	Asn	Gly	His	Arg	115	120	125	
Gly	Pro	Ser	Pro	Gly	Gln	Val	Lys	Tyr	Trp	Gln	Asn	Lys	Arg	Glu	Ile	130	135	140	
Pro	Asp	Pro	Asn	Glu	Asp	Tyr	Leu	Asp	Tyr	Val	His	Ala	Glu	Lys	Ser	145	150	155	160
Arg	Leu	Ala	Ser	Glu	Glu	Gln	Ile	Leu	Arg	Ala	Ala	Thr	Ser	Ile	Tyr	165	170	175	
Gly	Ala	Pro	Gly	Gln	Ala	Glu	Pro	Pro	Gln	Ala	Phe	Ile	Asp	Glu	Val	180	185	190	
Ala	Lys	Val	Tyr	Glu	Ile	Asn	His	Gly	Arg	Gly	Pro	Asn	Gln	Glu	Gln	195	200	205	
Met	Lys	Asp	Leu	Leu	Leu	Thr	Ala	Met	Glu	Met	Lys	His	Arg	Asn	Pro	210	215	220	
Arg	Arg	Ala	Leu	Pro	Lys	Pro	Lys	Pro	Lys	Pro	Asn	Ala	Pro	Thr	Gln	225	230	235	240
Arg	Pro	Pro	Gly	Arg	Leu	Gly	Arg	Trp	Ile	Arg	Thr	Val	Ser	Asp	Glu	245	250	255	
Asp	Leu	Glu	Gly	Ser	Ile	Ala	Thr	Met	Val	Ser	Lys	Gly	Glu	Glu	Leu	260	265	270	

Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn  
 275 280 285  
 Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr  
 290 295 300  
 Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val  
 305 310 315 320  
 Pro Trp Pro Thr Leu Val Thr Thr Leu Thr Tyr Gly Val Gln Cys Phe  
 325 330 335  
 Ser Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala  
 340 345 350  
 Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp  
 355 360 365  
 Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu  
 370 375 380  
 Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn  
 385 390 395 400  
 Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr  
 405 410 415  
 Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile  
 420 425 430  
 Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln  
 435 440 445  
 Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His  
 450 455 460  
 Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg  
 465 470 475 480  
 Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu  
 485 490 495  
 Gly Met Asp Glu Leu Tyr Lys  
 500

<210> 9  
 <211> 33  
 <212> DNA  
 <213> Artificial sequence

<220> Synthetic DNA  
 <223> Oligo V primer

<400> 9  
 gcgcgaattc gatggcatca gaggtaaag aga

<210> 10  
 <211> 32

<212> DNA  
 <213> Artificial sequence  
  
 <220> Synthetic DNA  
 <223> Oligo VI primer  
  
 <400> 10  
 cgcggtatccc tcaaggtcct catcagagac gg 32  
  
 <210> 11  
 <211> 36  
 <212> DNA  
 <213> Artificial sequence  
  
 <220>  
 <223> Oligo CD8 A primer  
  
 <400> 11  
 aacgaggaca gttatgtccc aagcgcagaa caaata 36  
  
 <210> 12  
 <211> 36  
 <212> DNA  
 <213> Artificial sequence  
  
 <220>  
 <223> Oligo CD8 B primer  
  
 <400> 12  
 tatttggtct gcgcttgga cataactgtc ctcggt 36  
  
 <210> 13  
 <211> 5676  
 <212> DNA  
 <213> Artificial sequence  
  
 <220>  
 <223> Plasmid pFB/his-CD8-VP3  
  
 <220>  
 <221> promoter  
 <222> (1)..(129)  
 <223> Polyhedrin promoter  
  
 <220>  
 <221> CDS  
 <222> (147)..(1043)  
 <223> His-CD8-VP3 ORF  
  
 <220>  
 <221> CDS  
 <222> (222)..(257)  
 <223> His-CD8 ORF  
  
 <400> 13  
 atcatggaga taattaaaat gataaccatc tcgcaaataa ataagtattt tactgttttc 60  
  
 gtaacagttt tgtaataaaa aaacctataa atattccgga ttattcatat cgtcccacca 120

tctgggctgctg atctcgggtcc gaaacc atg tct tac tac cat cac cat cac cat	173
Met Ser Tyr Tyr His His His His His	
1 5	
cac gat tac gat atc cca acg acc gaa aac ctg tat ttt cag ggc ggc	221
His Asp Tyr Asp Ile Pro Thr Thr Glu Asn Leu Tyr Phe Gln Gly Ala	
10 15 20 25	
aac gag gac agt tat gtc cca agc gca gaa caa ata gcc gcc atg gct	269
Asn Glu Asp Ser Tyr Val Pro Ser Ala Glu Gln Ile Ala Ala Met Ala	
30 35 40	
gca tca gag ttc aaa gag acc ccc gaa ctc gag agt gcc gtc aga gca	317
Ala Ser Glu Phe Lys Glu Thr Pro Glu Leu Glu Ser Ala Val Arg Ala	
45 50 55	
atg gaa gca gca gcc aac gtg gac cca cta ttc caa tct gca ctc agt	365
Met Glu Ala Ala Ala Asn Val Asp Pro Leu Phe Gln Ser Ala Leu Ser	
60 65 70	
gtg ttc atg tgg ctg gaa gag aat ggg att gtg act gac atg gcc aac	413
Val Phe Met Trp Leu Glu Glu Asn Gly Ile Val Thr Asp Met Ala Asn	
75 80 85	
ttc gca ctc agc gac ccg aac gcc cat cgg atg cga aat ttt ctt gca	461
Phe Ala Leu Ser Asp Pro Asn Ala His Arg Met Arg Asn Phe Leu Ala	
90 95 100 105	
aac gca cca caa gca ggc agc aag tct caa agg gcc aag tac ggg aca	509
Asn Ala Pro Gln Ala Gly Ser Lys Ser Gln Arg Ala Lys Tyr Gly Thr	
110 115 120	
gca ggc tac gga gtg gag gct cgg ggc ccc aca cca gag gaa gca cag	557
Ala Gly Tyr Gly Val Glu Ala Arg Gly Pro Thr Pro Glu Glu Ala Gln	
125 130 135	
agg gaa aaa gac aca cgg atc tca aag aag atg gag acc atg ggc atc	605
Arg Glu Lys Asp Thr Arg Ile Ser Lys Lys Met Glu Thr Met Gly Ile	
140 145 150	
tac ttt gca aca cca gaa tgg gta gca ctc aat ggg cac cga ggg cca	653
Tyr Phe Ala Thr Pro Glu Trp Val Ala Leu Asn Gly His Arg Gly Pro	
155 160 165	
agc cca ggc cag gta aag tac tgg cag aac aaa cga gaa ata ccg gac	701
Ser Pro Gly Gln Val Lys Tyr Trp Gln Asn Lys Arg Glu Ile Pro Asp	
170 175 180 185	
cca aac gag gac tat cta gac tac gtg cat gca gag aag agc cgg ttg	749
Pro Asn Glu Asp Tyr Leu Asp Tyr Val His Ala Glu Lys Ser Arg Leu	
190 195 200	
gca tca gaa gaa caa atc cta agg gca gct acg tct atc tac ggg gct	797
Ala Ser Glu Glu Gln Ile Leu Arg Ala Ala Thr Ser Ile Tyr Gly Ala	
205 210 215	

cca gga cag gca gag cca ccc caa gct ttc ata gac gaa gtt gcc aaa	845
Pro Gly Gln Ala Glu Pro Pro Gln Ala Phe Ile Asp Glu Val Ala Lys	
220 225 230	
gtc tat gaa atc aac cat gga cgt ggc cca aac caa gaa cag atg aaa	893
Val Tyr Glu Ile Asn His Gly Arg Gly Pro Asn Gln Glu Gln Met Lys	
235 240 245	
gat ctg ctc ttg act gcg atg gag atg aag cat cgc aat ccc agg cgg	941
Asp Leu Leu Leu Thr Ala Met Glu Met Lys His Arg Asn Pro Arg Arg	
250 255 260 265	
gct cta cca aag ccc aag cca aaa ccc aat gct cca aca cag aga ccc	989
Ala Leu Pro Lys Pro Lys Pro Lys Pro Asn Ala Pro Thr Gln Arg Pro	
270 275 280	
cct ggt cgg ctg ggc cgc tgg atc agg acc gtc tct gat gag gac ctt	1037
Pro Gly Arg Leu Gly Arg Trp Ile Arg Thr Val Ser Asp Glu Asp Leu	
285 290 295	
gag tga ggatccggaa ttcaaaggcc tacgtcgacg agctcactag tcgcggccgc	1093
Glu	
tttcgaatct agagcctgca gtctcgaggc atgcggtacc aagcttgtcg agaagtacta	1153
gaggatcata atcagccata ccacatttgt agaggtttta cttgctttaa aaaacctccc	1213
acacctcccc ctgaacctga aacataaaat gaatgcaatt gttgttgta acttgtttat	1273
tgcagcttat aatggttaca aataaagcaa tagcatcaca aatttcacaa ataaagcatt	1333
tttttcaactg cattctagtt gtggtttgtc caaactcatc aatgtatctt atcatgtctg	1393
gatctgatca ctgatctgc ctaggagatc cgaaccagat aagtgaatc tagttccaaa	1453
ctattttgtc atttttaatt ttctgattag cttacgacgc tacacccagt tcccatctat	1513
tttgtcactc ttccctaaat aatccttaaa aactccattt ccacccctcc cagttcccaa	1573
ctattttgtc cgcccacagc ggggcatttt tcttctgtt atgtttttaa tcaaacatcc	1633
tgccaactcc atgtgacaaa ccgtcatctt cggctacttt ttctctgtca cagaatgaaa	1693
atttttctgt catctcttcg ttattaatgt ttgtaattga ctgaatatca acgcttattt	1753
gcagcctgaa tggcgaatgg gacgcgccct gtagcggcgc attaagcgcg gcgggtgtgg	1813
tggttacgcg cagcgtgacc gctacacttg ccagcgccct agcgcgcgct cctttcgctt	1873
tcttcccttc ctttctcgcc acgttcgccg gctttccccg tcaagctcta aatcgggggc	1933
tcccttttagg gttccgattt agtgctttac ggcacctga ccccaaaaaa cttgattagg	1993
gtgatggttc acgtagtggg ccacgcacct gatagacggt ttttgcacct ttgacgttgg	2053
agtccacgtt ctttaatagt ggactcttgt tccaaactgg aacaacactc aaccctatct	2113
cggtctattc ttttgattta taagggattt tgccgatttc ggcctatttg ttaaaaaatg	2173

agctgattta acaaaaattt aacgcgaatt ttaacaaaat attaacgttt acaatttcag	2233
gtggcacttt tcggggaaat gtgcgcggaa cccctatttg tttatttttc taaatacatt	2293
caaatatgta tccgctcatg agacaataac cctgataaat gcttcaataa tattgaaaaa	2353
ggaagagtat gagtattcaa catttccgtg tcgcccttat tccctttttt gcggcatttt	2413
gccttcctgt ttttgctcac ccagaaacgc tgggtgaaagt aaaagatgct gaagatcagt	2473
tgggtgcacg agtgggttac atcgaactgg atctcaacag cggtaagatc cttgagagtt	2533
ttcgccccga agaacgtttt ccaatgatga gcacttttaa agttctgcta tgtggcgcg	2593
tattatcccg tattgacgcc gggcaagagc aactcggtcg ccgcatacac tattctcaga	2653
atgacttggt tgagtactca ccagtcacag aaaagcatct tacggatggc atgacagtaa	2713
gagaattatg cagtgtgcc ataaccatga gtgataacac tgcggccaac ttacttctga	2773
caacgatcgg aggaccgaag gagctaaccg cttttttgca caacatgggg gatcatgtaa	2833
ctcgccttga tcgttgggaa ccggagctga atgaagccat accaaacgac gagcgtgaca	2893
ccacgatgcc tgtagcaatg gcaacaacgt tgcgcaaact attaaactggc gaactactta	2953
ctctagcttc ccggcaacaa ttaatagact ggatggaggc ggataaagtt gcaggaccac	3013
ttctgcgctc ggcccttccg gctggctggt ttattgctga taaatctgga gccggtgagc	3073
gtgggtctcg cggtatcatt gcagcactgg ggccagatgg taagccctcc cgtatcgtag	3133
ttatctacac gacggggagt caggcaacta tggatgaacg aaatagacag atcgtgaga	3193
taggtgcctc actgattaag catttgtaac tgtcagacca agtttactca tatatacttt	3253
agattgattt aaaacttcat ttttaattta aaaggatcta ggtgaagatc ctttttgata	3313
atctcatgac caaaatccct taacgtgagt ttctgttcca ctgagcgtca gaccccgtag	3373
aaaagatcaa aggatcttct tgagatcctt tttttctgcg cgtaatctgc tgcttgcaaa	3433
caaaaaaacc accgctacca gcggtggttt gtttgccgga tcaagagcta ccaactcttt	3493
ttccgaaggt aactggcttc agcagagcgc agataccaaa tactgtcctt ctagtgtagc	3553
cgtagttagg ccaccacttc aagaactctg tagcaccgcc tacatacctc gctctgctaa	3613
tcctgttacc agtggctgct gccagtggcg ataagtcgtg tcttaccggg ttggactcaa	3673
gacgatagtt accggataag gcgcagcggc cgggctgaac ggggggttcg tgcacacagc	3733
ccagcttgga gcgaacgacc tacacgaac tgagatacct acagcgtgag cattgagaaa	3793
gcgccacgct tcccgaaggg agaaaggcgg acaggtatcc ggtaagcggc agggtcggaa	3853
caggagagcg cacgaggag cttccagggg gaaacgcctg gtatctttat agtcctgtcg	3913
ggtttgcgca cctctgactt gagcgtcgat ttttgtgatg ctcgtcaggg gggcggagcc	3973

tatggaaaaa cgccagcaac gcggcctttt tacggttcct ggccttttgc tggccttttg 4033  
ctcacatgtt ctttcctgcy ttatcccctg attctgtgga taaccgtatt accgcctttg 4093  
agtgagctga taccgctcgc cgcagccgaa cgaccgagcg cagcgagtca gtgagcgagg 4153  
aagcggaaga gcgcctgatg cggatatttc tccttacgca tctgtgcggt atttcacacc 4213  
gcagaccagc cgcgtaacct ggcaaaatcg gttacgggtg agtaataaat ggatgccttg 4273  
cgtaagcggg tgtgggcyga caataaagtc ttaaactgaa caaaatagat cttaaactatg 4333  
acaataaagt cttaaactag acagaatagt tgtaaactga aatcagtcga gttatgctgt 4393  
gaaaaagcat actggacttt tgttatggct aaagcaaact cttcattttc tgaagtgcaa 4453  
attgcccgtc gtattaaaga ggggcgtggc caagggcatg gtaaagacta tattcgcggc 4513  
gttgtgacaa tttaccgaac aactccgcgg ccgggaagcc gatctcggct tgaacgaatt 4573  
gttaggtggc ggtacttggg tcgatatcaa agtgcatac ttcttccgt atgcccact 4633  
ttgtatagag agccactgcy ggatcgtaac cgtaatctgc ttgcacgtag atcacataag 4693  
caccaagcgc gttggcctca tgcttgagga gattgatgag cgcggtggca atgcccgtcc 4753  
tccggtgctc gccggagact gcgagatcat agatatagat ctactacgc ggctgctcaa 4813  
acctgggcag aacgtaagcc gcgagagcgc caacaaccgc ttcttggtcg aaggcagcaa 4873  
gcgcgatgaa tgtcttacta cggagcaagt tcccgaggta atcggagtcc ggctgatgtt 4933  
gggagtaggt ggctacgtct ccgaactcac gaccgaaaag atcaagagca gcccgcatgg 4993  
atttgacttg gtcagggcgc agcctacatg tgcgaatgat gcccatactt gagccacct 5053  
actttgtttt agggcgactg ccctgctgcy taacatcggt gctgctgcgt aacatcggtg 5113  
ctgctccata acatcaaaca tcgaccacgc gcgtaacgcg cttgctgctt ggatgcccga 5173  
ggcatagact gtacaaaaaa acagtcataa caagccatga aaaccgccac tgcgccgtta 5233  
ccaccgctgc gttcggtcaa ggttctggac cagttgcgtg agcgcatacgc ctacttgcat 5293  
tacagtttac gaaccgaaca ggcttatgtc aactgggttc gtgccttcac ccgtttccac 5353  
ggtgtgcgtc acccggaac cttgggcagc agcgaagtcg aggcatttct gtccgtggctg 5413  
gcgaacgagc gcaaggtttc ggtctccacg catcgtcagg cattggcggc cttgctgttc 5473  
ttctacggca aggtgctgtg cacggatctg ccctggcttc aggagatcgg aagacctcgg 5533  
ccgtcgcggc gcttgccggt ggtgctgacc ccggatgaag tggttcgcat cctcggtttt 5593  
ctggaaggcg agcatcggtt gttcgcccag gactctagct atagttctag tggttggcta 5653  
cgtatactcc ggaatattaa tag 5676



<210> 14  
 <211> 298  
 <212> PRT  
 <213> Artificial sequence

<220>  
 <223> his-CD8-VP3 protein

<400> 14

Met	Ser	Tyr	Tyr	His	His	His	His	His	His	Asp	Tyr	Asp	Ile	Pro	Thr	1	5	10	15
Thr	Glu	Asn	Leu	Tyr	Phe	Gln	Gly	Ala	Asn	Glu	Asp	Ser	Tyr	Val	Pro	20	25	30	
Ser	Ala	Glu	Gln	Ile	Ala	Ala	Met	Ala	Ala	Ser	Glu	Phe	Lys	Glu	Thr	35	40	45	
Pro	Glu	Leu	Glu	Ser	Ala	Val	Arg	Ala	Met	Glu	Ala	Ala	Ala	Asn	Val	50	55	60	
Asp	Pro	Leu	Phe	Gln	Ser	Ala	Leu	Ser	Val	Phe	Met	Trp	Leu	Glu	Glu	65	70	75	80
Asn	Gly	Ile	Val	Thr	Asp	Met	Ala	Asn	Phe	Ala	Leu	Ser	Asp	Pro	Asn	85	90	95	
Ala	His	Arg	Met	Arg	Asn	Phe	Leu	Ala	Asn	Ala	Pro	Gln	Ala	Gly	Ser	100	105	110	
Lys	Ser	Gln	Arg	Ala	Lys	Tyr	Gly	Thr	Ala	Gly	Tyr	Gly	Val	Glu	Ala	115	120	125	
Arg	Gly	Pro	Thr	Pro	Glu	Glu	Ala	Gln	Arg	Glu	Lys	Asp	Thr	Arg	Ile	130	135	140	
Ser	Lys	Lys	Met	Glu	Thr	Met	Gly	Ile	Tyr	Phe	Ala	Thr	Pro	Glu	Trp	145	150	155	160
Val	Ala	Leu	Asn	Gly	His	Arg	Gly	Pro	Ser	Pro	Gly	Gln	Val	Lys	Tyr	165	170	175	
Trp	Gln	Asn	Lys	Arg	Glu	Ile	Pro	Asp	Pro	Asn	Glu	Asp	Tyr	Leu	Asp	180	185	190	
Tyr	Val	His	Ala	Glu	Lys	Ser	Arg	Leu	Ala	Ser	Glu	Glu	Gln	Ile	Leu	195	200	205	
Arg	Ala	Ala	Thr	Ser	Ile	Tyr	Gly	Ala	Pro	Gly	Gln	Ala	Glu	Pro	Pro	210	215	220	
Gln	Ala	Phe	Ile	Asp	Glu	Val	Ala	Lys	Val	Tyr	Glu	Ile	Asn	His	Gly	225	230	235	240
Arg	Gly	Pro	Asn	Gln	Glu	Gln	Met	Lys	Asp	Leu	Leu	Leu	Thr	Ala	Met	245	250	255	

Glu Met Lys His Arg Asn Pro Arg Arg Ala Leu Pro Lys Pro Lys Pro  
260 265 270

Lys Pro Asn Ala Pro Thr Gln Arg Pro Pro Gly Arg Leu Gly Arg Trp  
275 280 285

Ile Arg Thr Val Ser Asp Glu Asp Leu Glu  
290 295

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> IPC 7 C12N7/04 A61K39/12 C12N15/62 C07K14/08 C07K19/00 C12N15/87 C12N15/86 C12N5/10		
According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) IPC 7 C12N		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used) EPO-Internal, WPI Data, BIOSIS, MEDLINE		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEVALIER C ET AL: "The maturation process of pVP2 requires assembly of infectious bursal disease virus capsids" JOURNAL OF VIROLOGY, THE AMERICAN SOCIETY FOR MICROBIOLOGY, US, vol. 76, no. 5, March 2002 (2002-03), pages 2384-2392, XP002218366 ISSN: 0022-538X the whole document	1-4, 6, 9-14, 17-20, 23-29
X	WO 02/088339 A (INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE ; DELMAS, BERNARD; CHEVA) 7 November 2002 (2002-11-07)  page 2 - page 3 page 9 - page 10; claims 1-14  ----- -/--	1-4, 6, 9-14, 17-20, 23-29
<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C.		
<input checked="" type="checkbox"/> Patent family members are listed in annex.		
* Special categories of cited documents : *A* document defining the general state of the art which is not considered to be of particular relevance *E* earlier document but published on or after the international filing date *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) *O* document referring to an oral disclosure, use, exhibition or other means *P* document published prior to the international filing date but later than the priority date claimed *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. *&* document member of the same patent family		
Date of the actual completion of the international search  15 June 2005		Date of mailing of the international search report  11/07/2005
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016		Authorized officer  Paresce, D

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>HU Y ET AL: "Chimeric infectious bursal disease virus-like particles expressed in insect cells and purified by immobilized metal affinity chromatography"  BIOTECHNOLOGY AND BIOENGINEERING.  INCLUDING: SYMPOSIUM BIOTECHNOLOGY IN ENERGY PRODUCTION AND CONSERVATION, JOHN WILEY &amp; SONS. NEW YORK, US,  vol. 63, no. 6, 20 June 1999 (1999-06-20),  pages 721-729, XP002190336  ISSN: 0006-3592  page 721 - page 724</p>	1-29
Y	<p>US 5 788 970 A (VAKHARIA ET AL)  4 August 1998 (1998-08-04)  cited in the application  columns 14-15</p>	1-29
A	<p>FERNÁNDEZ-ARIAS A ET AL: "Expression of ORF A1 of infectious bursal disease virus results in the formation of virus-like particles"  JOURNAL OF GENERAL VIROLOGY, SOCIETY FOR GENERAL MICROBIOLOGY, READING, GB,  vol. 79, no. part 5, May 1998 (1998-05),  pages 1047-1054, XP002218365  ISSN: 0022-1317  cited in the application  page 1049 - page 1053</p>	1-29
A	<p>MARTINEZ-TORRECUADRADA J L ET AL:  "Different Architectures in the Assembly of Infectious Bursal Disease Virus Capsid Proteins Expressed in Insect Cells"  VIROLOGY, ACADEMIC PRESS, ORLANDO, US,  vol. 278, no. 2,  20 December 2000 (2000-12-20), pages  322-331, XP004435746  ISSN: 0042-6822  cited in the application  the whole document</p>	1-29
A	<p>MARTINEZ-TORRECUADRADA J L ET AL:  "Structure-dependent efficacy of infectious bursal disease virus (IBDV) recombinant vaccines"  VACCINE, BUTTERWORTH SCIENTIFIC.  GUILDFORD, GB,  vol. 21, no. 23, 4 July 2003 (2003-07-04),  pages 3342-3350, XP004429746  ISSN: 0264-410X  the whole document</p>	1-29

Patent document cited in search report		Publication date		Patent family member(s)	Publication date
WO 02088339	A	07-11-2002	FR	2824327 A1	08-11-2002
			WO	02088339 A2	07-11-2002
<hr/>					
US 5788970	A	04-08-1998	AU	696656 B2	17-09-1998
			AU	2129195 A	17-10-1995
			CA	2186856 A1	05-10-1995
			EP	0755259 A1	29-01-1997
			JP	9510873 T	04-11-1997
			US	6017759 A	25-01-2000
			WO	9526196 A1	05-10-1995
			US	6156314 A	05-12-2000
<hr/>					